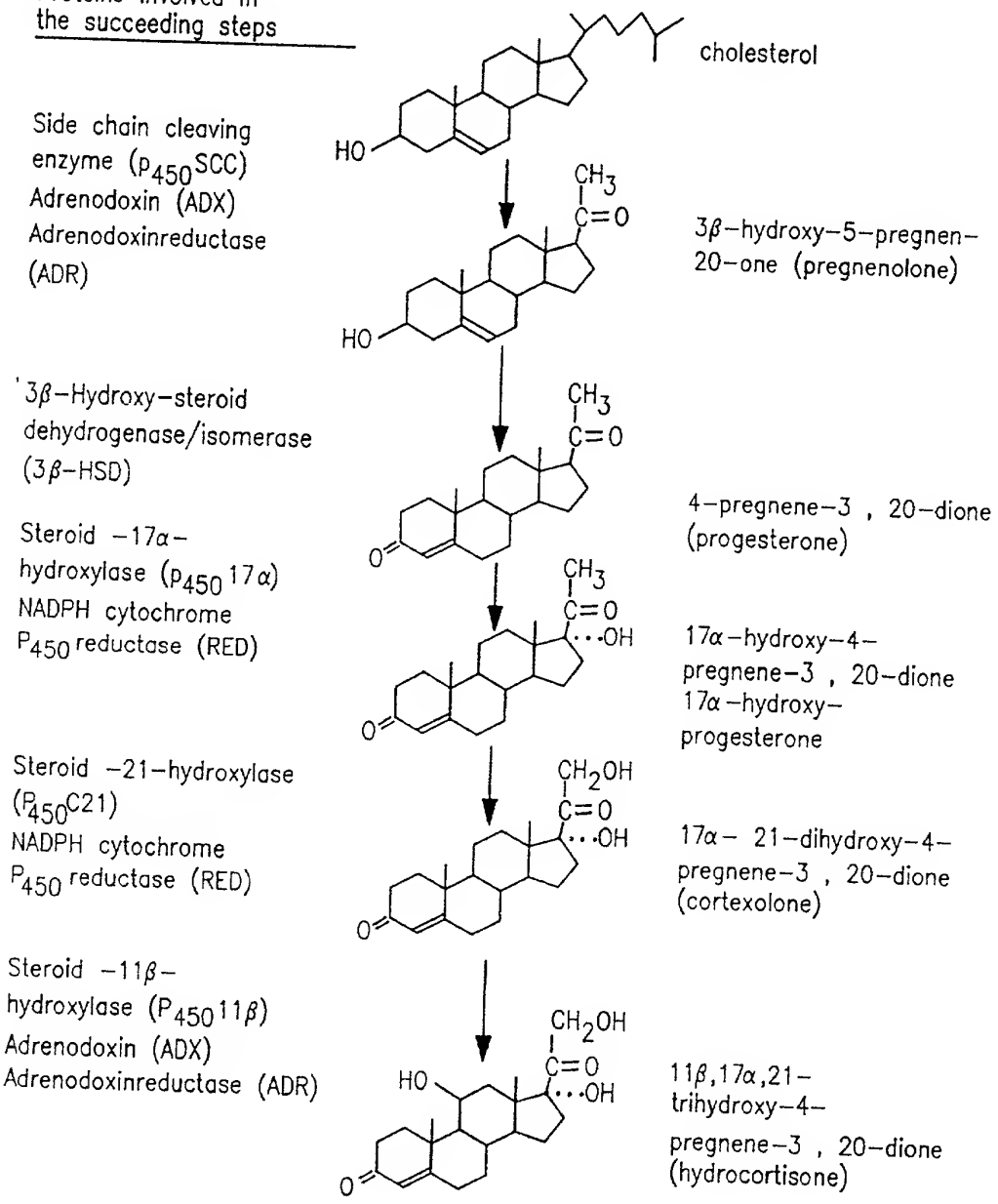
Proteins involved in the succeeding steps
cholesterol
Side chain cleaving enzyme (P_{450}SCC)
Adrenodoxin (ADX)
Adrenodoxinreductase (ADR)
3β-hydroxy-5-pregnen-20-one (pregnenolone)
3β-Hydroxy-steroid dehydrogenase/isomerase (3β-HSD)
4-pregnene-3 , 20-dione (progesterone)
Steroid -17α-hydroxylase (P_{450} 17α)
NADPH cytochrome P_{450} reductase (RED)
17α-hydroxy-4-pregnene-3 , 20-dione
17α-hydroxy-progesterone
Steroid -21-hydroxylase (P_{450}C21)
NADPH cytochrome P_{450} reductase (RED)
17α- 21-dihydroxy-4-pregnene-3 , 20-dione (cortexolone)
Steroid -11β-hydroxylase (P_{450}11β)
Adrenodoxin (ADX)
Adrenodoxinreductase (ADR)
11β,17α,21-trihydroxy-4-pregnene-3 , 20-dione (hydrocortisone)

FIG. 1

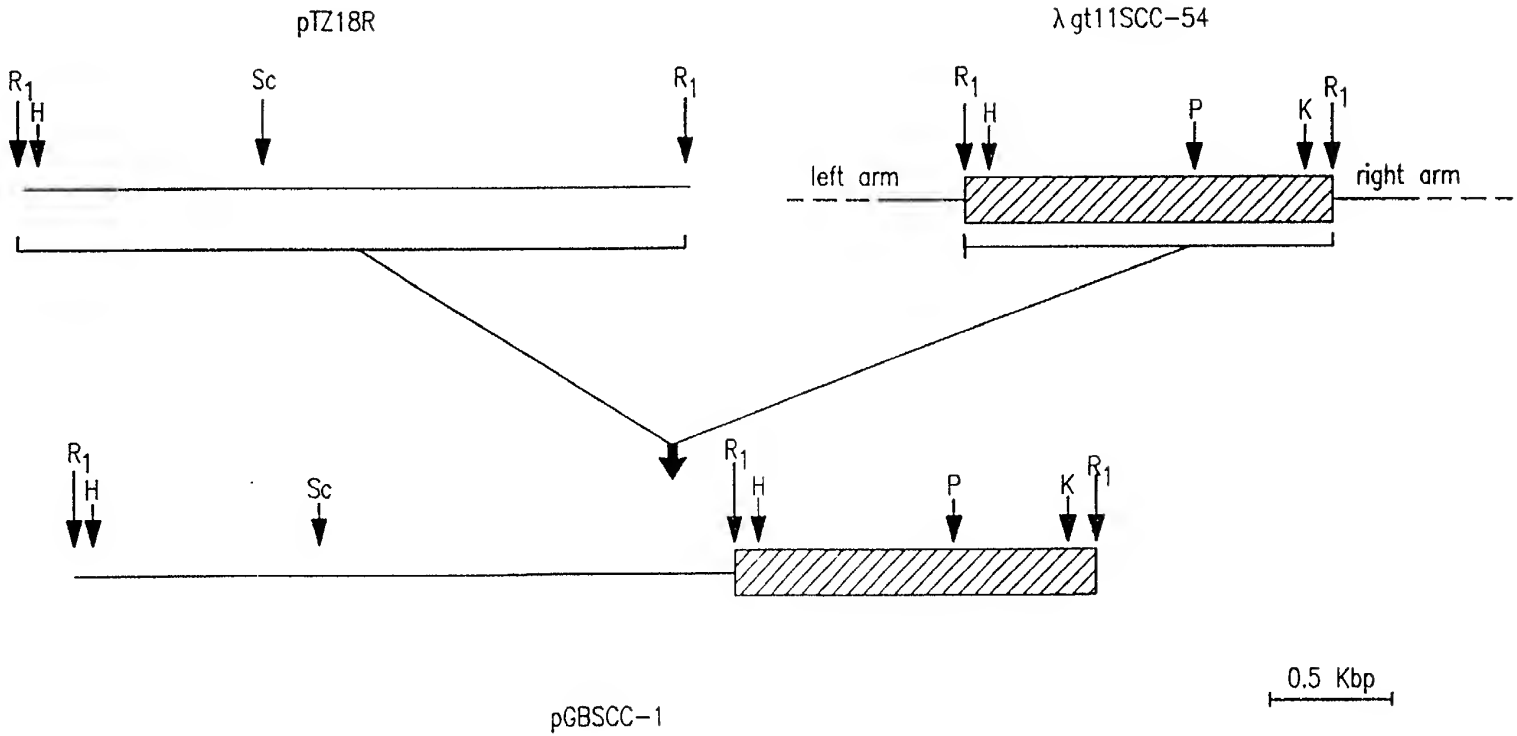
pTZ18R
λgt11SCC-54
R1
H
Sc
R1
R1
H
P
K
R1
left arm
right arm
R1
H
Sc
R1
H
P
K
R1
0.5 Kbp
pGBSCC-1

FIG. 2

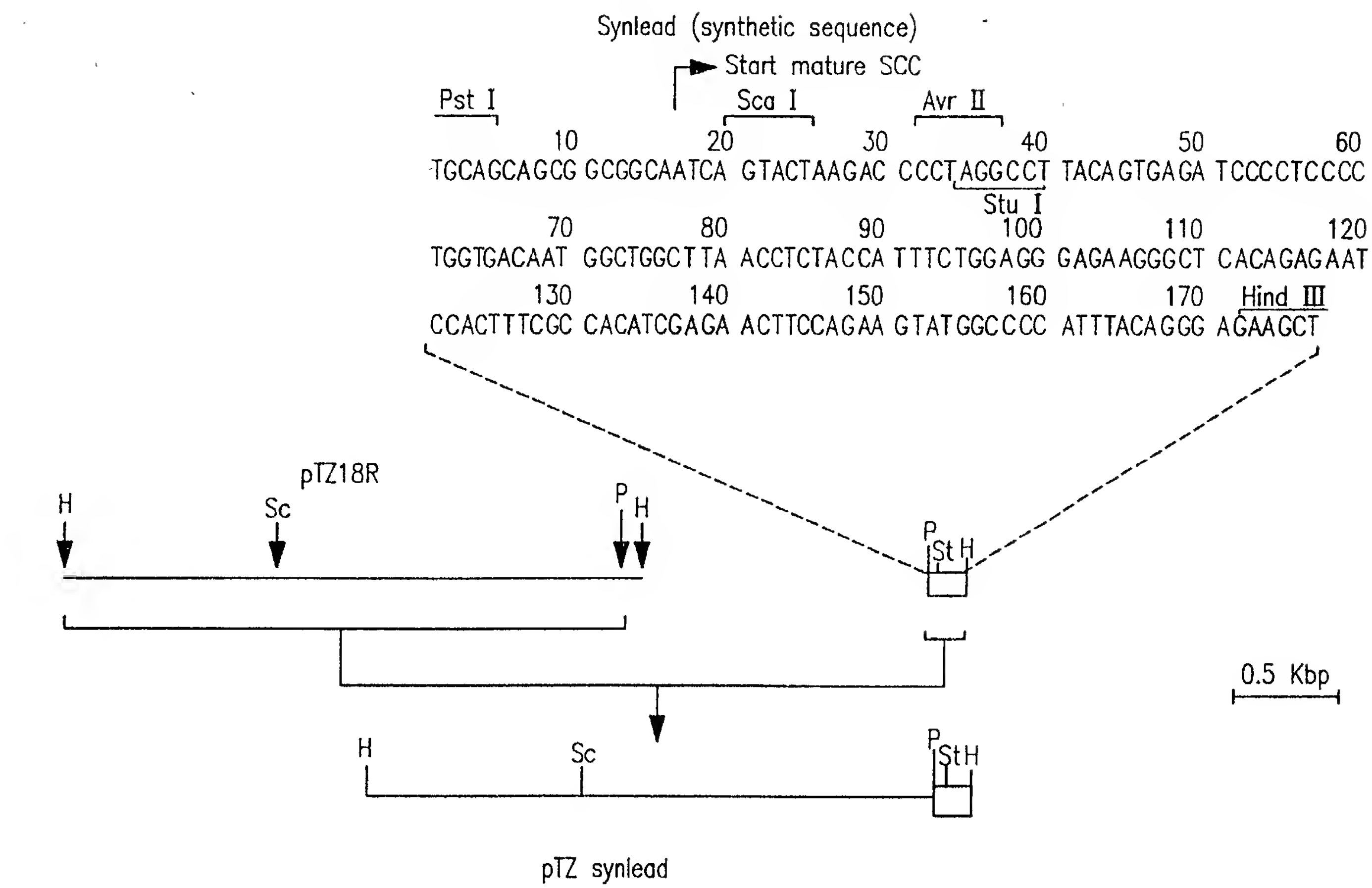
Synlead (synthetic sequence)
Start mature SCC
Pst I
Sca I
Avr II
10 20 30 40 50 60
TGCAGCAGCG GCGGCAATCA GTACTAAGAC CCCTAGGCCT TACAGTGAGA TCCCCTCCCC
Stu I
70 80 90 100 110 120
TGGTGACAAT GGCTGGCTTA ACCTCTACCA TTTCTGGAGG GAGAAGGGCT CACAGAGAAT
130 140 150 160 170 Hind III
CCACTTTCGC CACATCGAGA ACTTCCAGAA GTATGGCCCC ATTTACAGGG AGAAGCT
pTZ18R
H
Sc
P
H
P
St
H
0.5 Kbp
H
Sc
P
St
H
pTZ synlead

FIG. 3

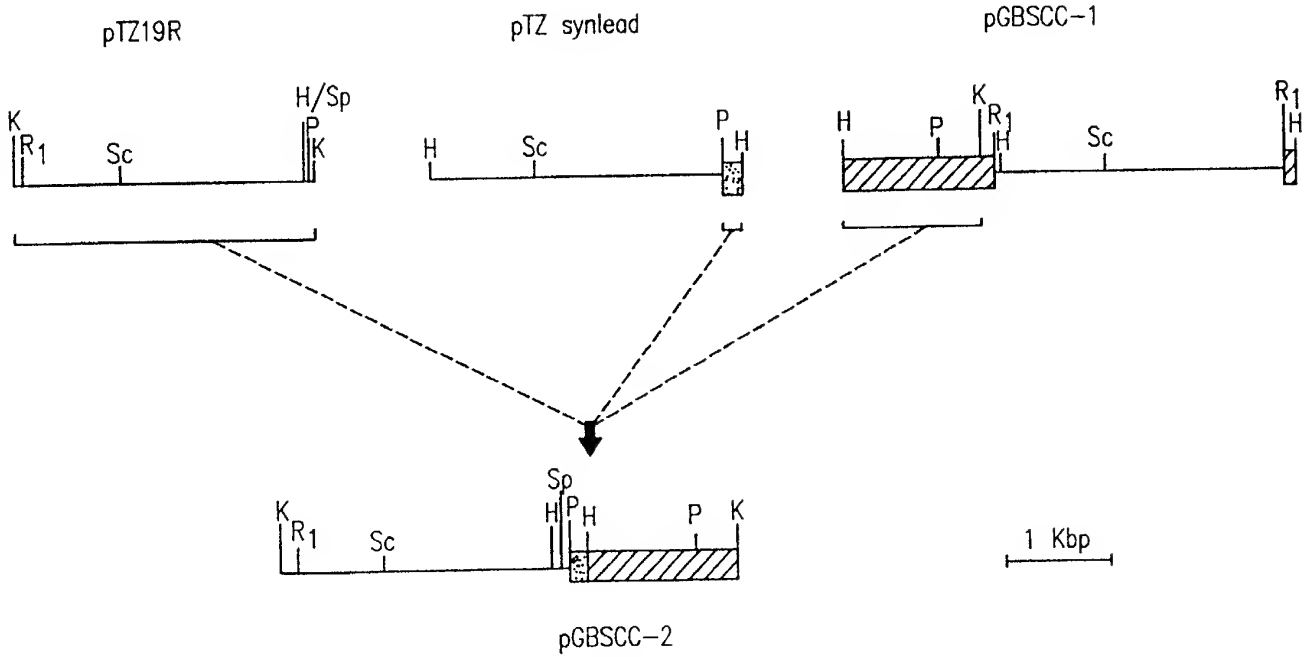

FIG. 4

```
                                        30                                      60                                      90
AATTCACCTC GAAAGGAAGC TGATAAACCG ATACAATTAA AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG ATTTTCAACG TGAAAAAATT

                                       120                                     150                                     180
ATTATTCGCA ATTCCAAGCT AATTCACCTC GAAAGCAAGC TGATAAACCG ATACAATTAA AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG

                                       210                                     240                                     270
ATTTTCAACG TGAAAAAATT ATTATTCGCA ATTCCAAGCT CTGCCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC

                                       300                                     330                                     360
TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCAGA TCACGCGCCC TGTAGCGGCG CATTAAGCGC GGCGGGTGTG GTGGTTACGC

                                       390                                     420                                     450
GCAGCGTGAC CGCTACACTT GCCAGCGCCC TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT CCTTTCTCGC CACGTTCGCC GGCTTTCCCC

                                       480                                     510                                     540
GTCAAGCTCT AAATCGGGGG CTCCCTTTAG GGTTCCGATT TAGTGCTTTA CGGCACCTCG ACCCCAAAAA ACTTGATTAG GGTGATGGTT

                                       570                                     600                                     630
CACGTAGTGG GCCATCGCCC TGATAGACGG TTTTTCGCCC TTTGACGTTG GAGTCCACGT TCTTTAATAG TGGACTCTTG TTCCAAACTG

                                       660                                     690                                     720
GAACAACACT CAACCCTATC TCGGTCTATT CTTTTGATTT ATAAGGGATT TTGCCGATTT CGGCCTATTG GTTAAAAAAT GAGCTGATTT

                                       750                                     780                                     810
AACAAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT TACAATTTGA TCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT

                                       840                                     870                                     900
CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC

                                       930                                     960                                     990
CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA

                                      1020                                    1050                                    1080
AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
```

FIG. 5A

```
                              1110                                    1140                                    1170
CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG
                              1200                                    1230                                    1260
CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC
                              1290                                    1320                                    1350
TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC
                              1380                                    1410                                    1440
TACGGCTACA CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC
                              1470                                    1500                                    1530
AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC
                              1560                                    1590                                    1620
TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC
                              1650                                    1680                                    1710
CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA
                              1740                                    1770                                    1800
CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT
                              1830                                    1860                                    1890
GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC
                              1920                                    1950                                    1980
AGAAGTGGTC CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG
                              2010                                    2040                                    2070
CGCAACGTTG TTGCCATTGC TGCAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG
```

FIG. 5B

```
                              2100                                                     2130                                                     2160
CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA
                              2190                                                     2220                                                     2250
TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC TTTTCTGTGA CTGGTGAGTA CTCAACCAAG
                              2280                                                     2310                                                     2340
TCATTCTGAG AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC AACACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA
                              2370                                                     2400                                                     2340
GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC CACTCGTGCA
                              2460                                                     2490                                                     2520
CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG
                              2550                                                     2580                                                     2610
GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG AAGCAGACAG TTTTATTGTT CATGATGATA TATTTTTATC
                              2640                                                     2670                                                     2700
TTGTGCAATG TAACATCAGA GATTTTGAGA CACAACGTGG CTTTGTTGAA TAAATCGAAC TTTTGCTGAG TTGACTCCCC GCGCGCGATG
                              2730                                                     2760                                                     2790
GGTCGAATTT GCTTTCGAAA AAAAAGCCCG CTCATTAGGC GGGCTAAAAA AAAGCCCGCT CATTAGGCGG GCTCGAATTT CTGCCATTCA
                              2820                                                     2850                                                     2880
TCCGCTTATT ATCACTTATT CAGGCGTAGC AACCAGGCGT TTAAGGGCAC CAATAACTGC CTTAAAAAAA TTACGCCCCG CCCTGCCACT
                              2910                                                     2940                                                     2970
CATCGCAGTA CTGTTGTAAT TCATTAAGCA TTCTGCCGAC ATGGAAGCCA TCACAGACGG CATGATGAAC CTGAATCGCC AGCGGCATCA
```

FIG. 5C

```
                                3000                                 3030                                 3060
GCACCTTGTC GCCTTGCGTA TAATATTTGC CCATAGTGAA AACGGGGGCG AAGAAGTTGT CCATATTCGC CACGTTTAAA TCAAAACTGG
                                3090                                 3120                                 3150
TGAAACTCAC CCAGGGATTG GCTGAGACGA AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA
                                3180                                 3210                                 3240
CATCTTGCGA ATATATGTGT AGAAACTGCC GGAAATCGTC GTGGTATTCA CTCCAGAGCG ATGAAAACGT TTCAGTTTGC TCATGGAAAA
                                3270                                 3300                                 3330
CGGTGTAACA AGGGTGAACA CTATCCCATA TCACCAGCTC ACCGTCTTTC ATTGCCATAC GAAATTCCGG ATGAGCATTC ATCAGGCGGG
                                3360                                 3390                                 3420
CAAGAATGTG AATAAAGGCC GGATAAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA AGGCCGTAAT ATCCAGCTAA ACGGTCTGGT
                                3450                                 3480                                 3510
TATAGGTACA TTGAGCAACT GACTGAAATG CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT
                                3540                                 3570                                 3600
TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCG GTAGTGATCT TATTTCATTA TGGTGAAAGT
                                3630                                 3660                                 3690
TGGAACCTCT TACGTGCCGA TCAACGTCTC ATTTTCGCCA AAAGTTGGCC CAGGGCTTCC CGGTATCAAC AGGGACACCA GGATTTATTT
                                3720                                 3750                                 3780
ATTCTGCGAA GTGATCTTCC GTCACAGGTA TTTATTCGAA GACGAAAGGG CATCGCGCGC GGGGAATTCC CGGGAGAGCT CGATATCGCA
                                3810                                 3840                                 3870
TGCGGTACCT CTAGAAGAAG CTTGGAGACA AGGTAAAGGA TAAAACAGCA CAATTCCAAG AAAAACACGA TTTAGAACCT AAAAAGAACG
                                3900                                 3930                                 3960
AATTTGAACT AACTCATAAC CGAGAGGTAA AAAAAGAACG AAGTCGAGAT CAGGGAATGA GTTTATAAAA TAAAAAAAGC ACCTGAAAAG
```

FIG. 5D

```
                                 3990                                4020                                4050
GTGTCTTTTT TTGATGGTTT TGAACTTGTT CTTTCTTATC TTGATACATA TAGAAATAAC GTCATTTTTA TTTTAGTTGC TGAAAGGTGC
                                 4080                                4110                                4140
GTTGAAGTGT TGGTATGTAT GTGTTTTAAA GTATTGAAAA CCCTTAAAAT TGGTTGCACA GAAAAACCCC ATCTGTTAAA GTTATAAGTG
                                 4170                                4200                                4230
ACTAAACAAA TAACTAAATA GATGGGGGTT TCTTTTAATA TTATGTGTCC TAATAGTAGC ATTTATTCAG ATGAAAAATC AAGGGTTTTA
                                 4260                                4290                                4320
GTGGACAAGA CAAAAAGTGG AAAAGTGAGA CCATGGAGAG AAAAGAAAAT CGCTAATGTT GATTACTTTG AACTTCTGCA TATTCTTGAA
                                 4350                                4380                                4410
TTTAAAAAGG CTGAAAGAGT AAAAGATTGT GCTGAAATAT TAGAGTATAA ACAAAATCGT GAAACAGGCG AAAGAAAGTT GTATCGAGTG
                                 4440                                4470                                4500
TGGTTTTGTA AATCCAGGCT TTGTCCAATG TGCAACTGGA GGAGAGCAAT GAAACATGGC ATTCAGTCAC AAAAGGTTGT TGCTGAAGTT
                                 4530                                4560                                4590
ATTAAACAAA AGCCAACAGT TCGTTGGTTG TTTCTCACAT TAACAGTTAA AAATGTTTAT GATGGCGAAG AATTAAATAA GAGTTTGTCA
                                 4620                                4650                                4680
GATATGGCTC AAGGATTTCG CCGAATGATG CAATATAAAA AAATTAATAA AAATCTTGTT GGTTTTATGC GTGCAACGGA AGTGACAATA
                                 4710                                4740                                4770
AATAATAAAG ATAATTCTTA TAATCAGCAC ATGCATGTAT TGGTATGTGT GGAACCAACT TATTTTAAGA ATACAGAAAA CTACGTGAAT
                                 4800                                4830                                4860
CAAAAACAAT GGATTCAATT TTGGAAAAAG GCAATGAAAT TAGACTATGA TCCAAATGTA AAAGTTCAAA TGATTCGACC GAAAAATAAA
                                 4890                                4920                                4950
TATAAATCGG ATATACAATC GGCAATTGAC GAAACTGCAA AATATCCTGT AAAGGATACG GATTTTATGA CCGATGATGA AGAAAAGAAT
                                 4980                                5010                                5040
TTGAAACGTT TGTCTGATTT GGAGGAAGGT TTACACCGTA AAAGGTTAAT CTCCTATGGT GGTTTGTTAA AAGAAATACA TAAAAAATTA
```

FIG. 5E

```
                              5070                                              5100                                              5130
AACCTTGATG ACACAGAAGA AGGCGATTTG ATTCATACAG ATGATGACGA AAAAGCCGAT GAAGATGGAT TTTCTATTAT TGCAATGTGG
                              5160                                              5190                                              5220
AATTGGGAAC GGAAAAATTA TTTTATTAAA GAGTAGTTCA ACAAACGGGC CAGTTTGTTG AAGATTAGAT GCTATAATTG TTATTAAAAG
                              5250                                              5280                                              5310
GATTGAAGGA TGCTTAGGAA GACGAGTTAT TAATAGCTGA ATAAGAACGG TGCTCTCCAA ATATTCTTAT TTAGAAAAGC AAATCTAAAA
                              5340                                              5370                                              5400
TTATCTGAAA AGGGAATGAG AATAGTGAAT GGACCAATAA TAATGACTAG AGAAGAAAGA ATGAAGATTG TTCATGAAAT TAAGGAACGA
                              5430                                              5460                                              5490
ATATTGGATA AATATGGGGA TGATGTTAAG GCTATTGGTG TTTATGGCTC TCTTGGTCGT CAGACTGATG GGCCCTATTC GGATATTGAG
                              5520                                              5550                                              5580
ATGATGTGTG TCATGTCAAC AGAGGAAGCA GAGTTCAGCC ATGAATGGAC AACCGGTGAG TGGAAGGTGG AAGTGAATTT TGATAGCGAA
                              5610                                              5640                                              5670
GAGATTCTAC TAGATTATGC ATCTCAGGTG GAATCAGATT GGCCGCTTAC ACATGGTCAA TTTTTCTCTA TTTTGCCGAT TTATGATTCA
                              5700                                              5730                                              5760
GGTGGATACT TAGAGAAAGT GTATCAAACT GCTAAATCGG TAGAAGCCCA AACGTTCCAC GATGCGATTT GTGCCCTTAT CGTAGAAGAG
                              5790                                              5820                                              5850
CTGTTTGAAT ATGCAGGCAA ATGGCGTAAT ATTCGTGTGC AAGGACCGAC AACATTTCTA CCATCCTTGA CTGTACAGGT AGCAATGGCA
                              5880                                              5910                                              5940
GGTGCCATGT TGATTGGTCT GCATCATCGC ATCTGTTATA CGACGAGCGC TTCGGTCTTA ACTGAAGCAG TTAAGCAATC AGATCTTCCT
                              5970                                              6000                                              6030
TCAGGTTATG ACCATCTGTG CCAGTTCGTA ATGTCTGGTC AACTTTCCGA CTCTGAGAAA CTTCTGGAAT CGCTAGAGAA TTTCTGGAAT
                              6060                                              6090                                              6120
GGGATTCAGG AGTGGACAGA ACGACACGGA TATATAGTGG ATGTGTCAAA ACGCATACCA TTTTGAACGA TGACCTCTAA TAATTGTTAA
```

FIG. 5F

```
                                            6150                                             6180                                             6210
TCATGTTGGT TACGTATTTA TTAACTTCTC CTAGTATTAG TAATTATCAT GGCTGTCATG GCGCATTAAC GGAATAAAGG GTGTGCTTAA

                                            6240                                             6270                                             6300
ATCGGGCCAT TTTGCGTAAT AAGAAAAAGG ATTAATTATG AGCGAATTGA ATTAATAATA AGGTAATAGA TTTACATTAG AAAATGAAAG

                                            6330                                             6360                                             6390
GGGATTTTAT GCGTGAGAAT GTTACAGTCT ATCCCGGCAT TGCCAGTCGG GGATATTAAA AAGAGTATAG GTTTTTATTG CGATAAACTA

                                            6420                                             6450                                             6480
GGTTTCACTT TGGTTCACCA TGAAGATGGA TTCGCAGTTC TAATGTGTAA TGAGGTTCGG ATTCATCTAT GGGAGGCAAG TGATGAAGGC

                                            6510                                             6540                                             6570
TGGCGCTCTC GTAGTAATGA TTCACCGGTT TGTACAGGTG CGGAGTCGTT TATTGCTGGT ACTGCTAGTT GCCGCATTGA AGTAGAGGGA

                                            6600                                             6630                                             6660
ATTGATGAAT TATATCAACA TATTAAGCCT TTGGGCATTT TGCACCCCAA TACATCATTA AAAGATCAGT GGTGGGATGA ACGAGACTTT

                                            6690                                             6720                                             6750
GCAGTAATTG ATCCCGACAA CAATTTGATT AGCTTTTTTC AACAAATAAA AAGCTAAAAT CTATTATTAA TCTGTTCAGC AATCGGGCGC

                                            6780                                             6810                                             6840
GATTGCTGAA TAAAAGATAC GAGAGACCTC TCTTGTATCT TTTTTATTTT GAGTGGTTTT GTCCGTTACA CTAGAAAACC GAAAGACAAT

                                            6870                                             6900                                             6930
AAAAATTTTA TTCTTGCTGA GTCTGGCTTT CGGTAAGCTA GACAAAACGG ACAAAATAAA AATTGGCAAG GGTTTAAAGG TGGAGATTTT

                                            6960                                             6990                                             7020
TTGAGTGATC TTCTCAAAAA ATACTACCTG TCCCTTGCTG ATTTTTAAAC GAGCACGAGA GCAAAACCCC CCTTTGCTGA GGTGGCAGAG

                                            7050                                             7080                                             7110
GGCAGGTTTT TTTGTTTCTT TTTTCTCGTA AAAAAAAGAA AGGTCTTAAA GGTTTTATGG TTTTGGTCGG CACTGCCGAC AGCCTCGCAG

                                            7140                                             7170                                             7200
GACACACACT TTATGAATAT AAAGTATAGT GTGTTATACT TTACTTGGAA GTGGTTGCCG GAAAGAGCGA AAATGCCTCA CATTTGTGCC

                                            7230                                             7260                                             7290
ACCTAAAAAG GAGCGATTTA CATATGAGTT ATGCAGTTTG TAGAATGCAA AAAGTGAAAT CAGGGGGATC CTCTAGAGTC GAGCTCAAGC

                                            7320
TAGCTTGGTA CGTACCAGAT CTGAGATCAC GCGTTCTAGA GGTCGA
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FIG. 5G

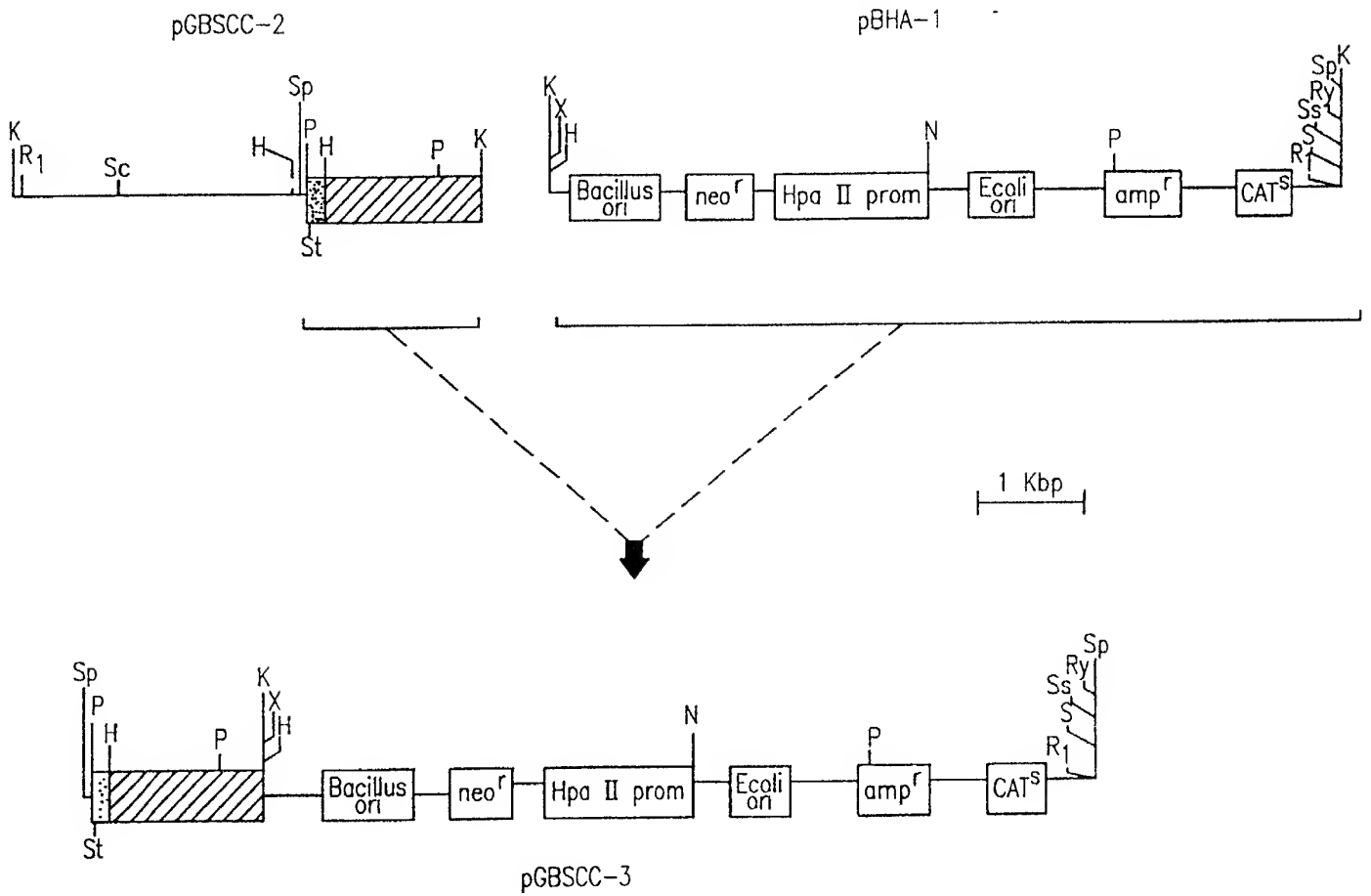
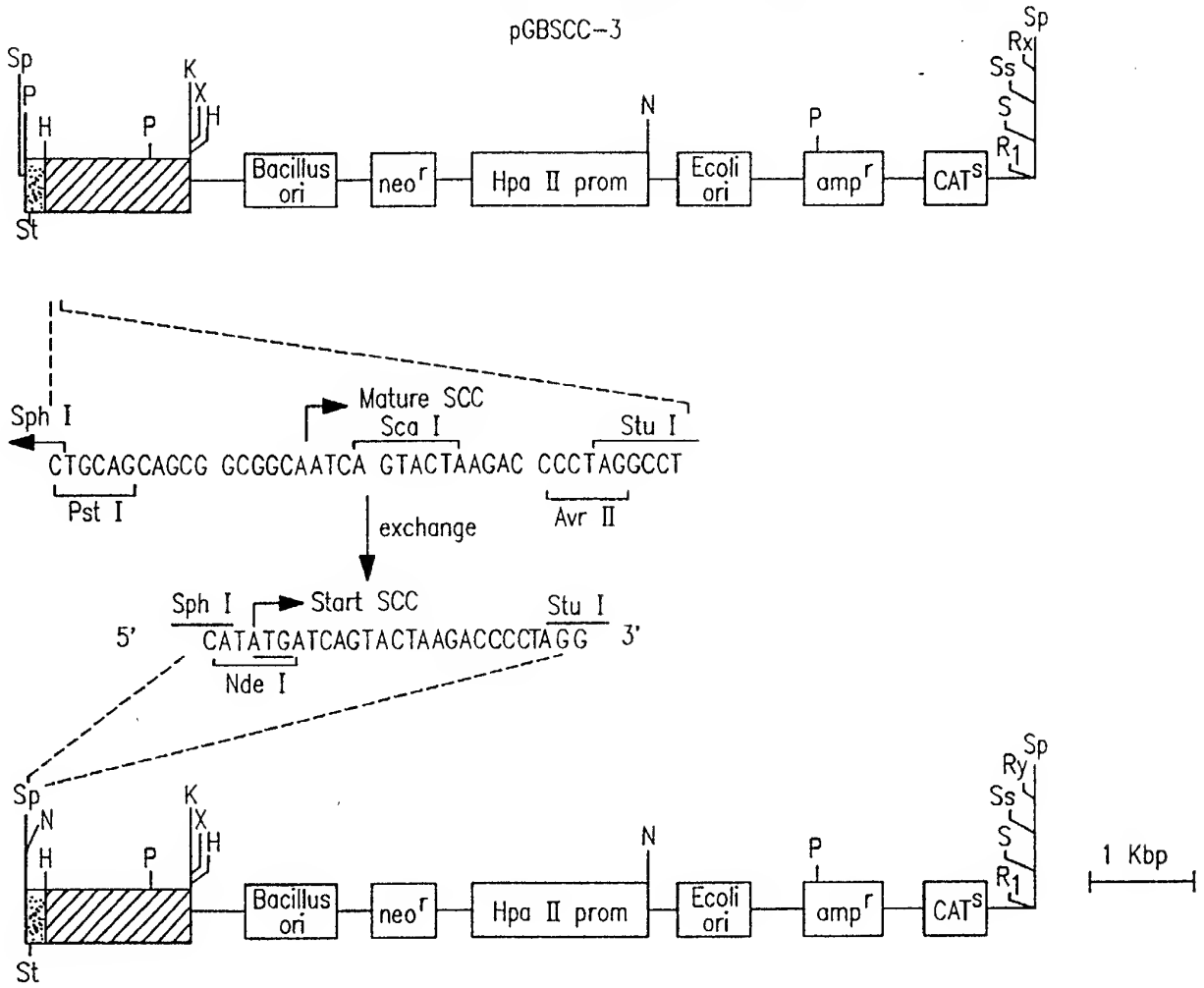

pGBSCC-2
pBHA-1
K
R1
Sc
H
Sp
P
H
P
K
St
K
X
H
N
P
Sp
K
Ry
Ss
S
R
Bacillus ori
neo^r
Hpa II prom
Ecoli ori
amp^r
CAT^s
1 Kbp
Sp
P
H
P
K
X
H
St
Bacillus ori
neo^r
Hpa II prom
N
Ecoli ori
P
amp^r
CAT^s
Sp
Ry
Ss
S
R1
pGBSCC-3

FIG. 6



pGBSCC-4

FIG. 7

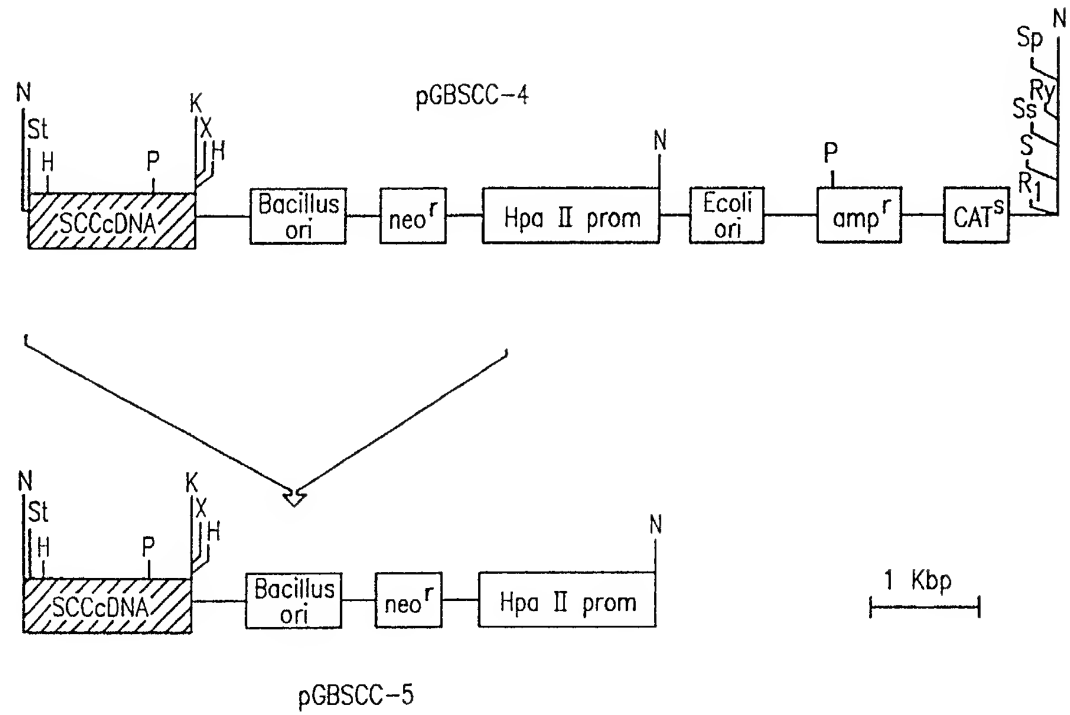

pGBSCC-4
N
St
H
P
K
X
H
SCCcDNA
Bacillus ori
neo^r
Hpa II prom
N
Ecoli ori
P
amp^r
CAT^s
Sp
N
Ry
Ss
S
R1
pGBSCC-5
1 Kbp

FIG. 8

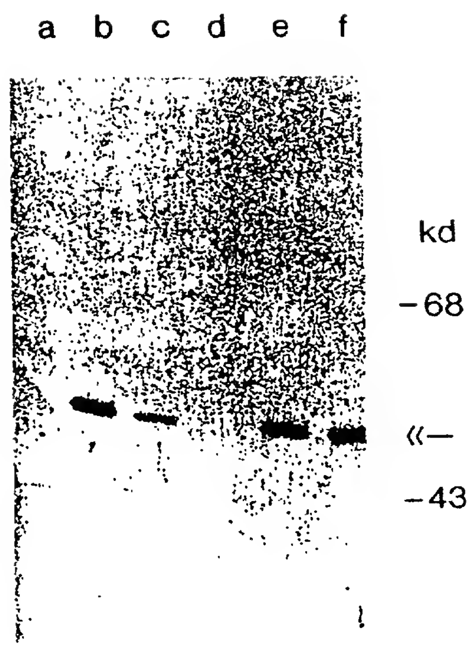


FIG. 9

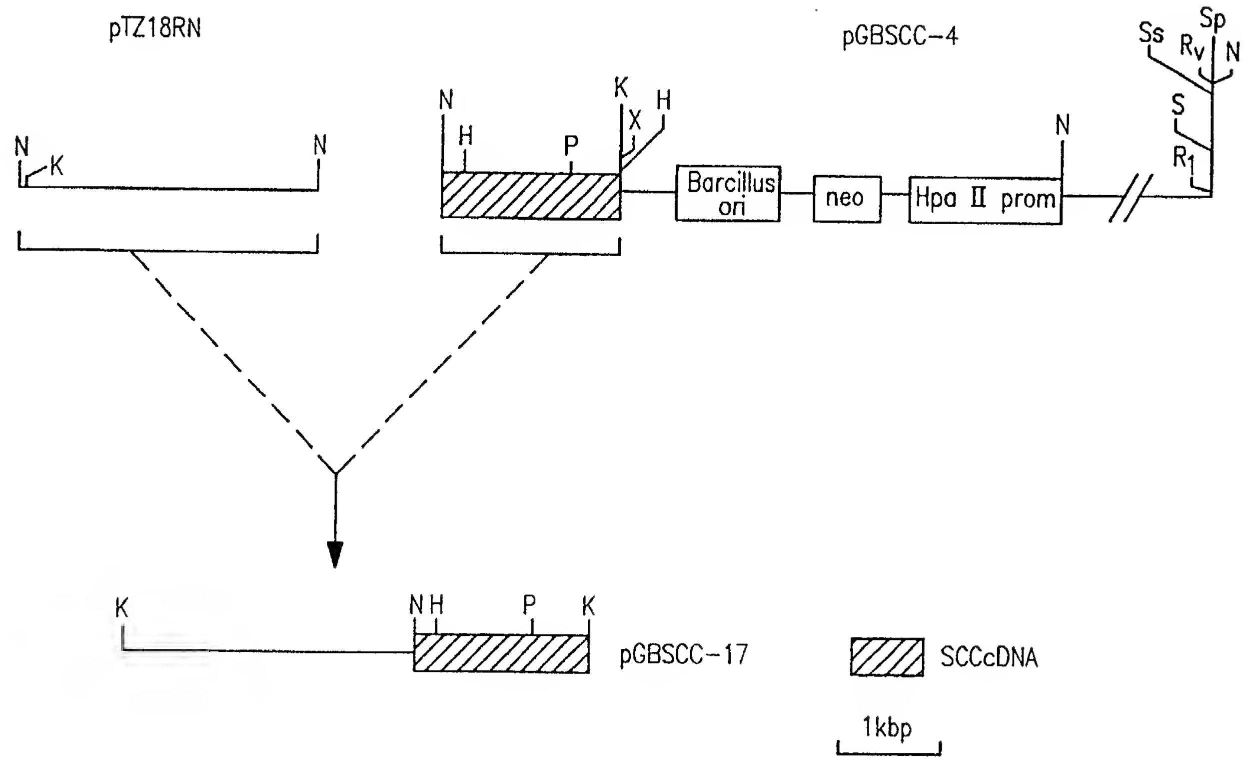

pTZ18RN
N
K
N
pGBSCC-4
N
H
P
K
X
H
Barcillus ori
neo
Hpa II prom
N
Ss
Sp
Rv
N
S
R1
K
N H
P
K
pGBSCC-17
SCCcDNA
1kbp

FIG. 10

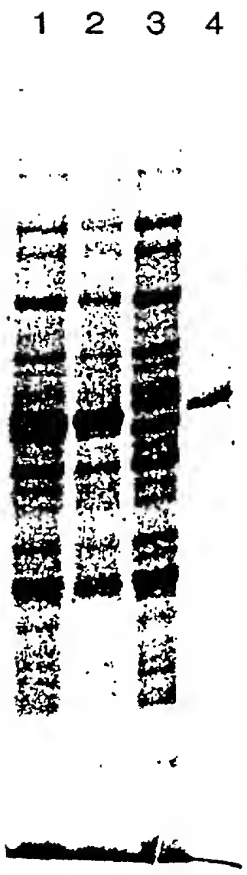


FIG. IIA

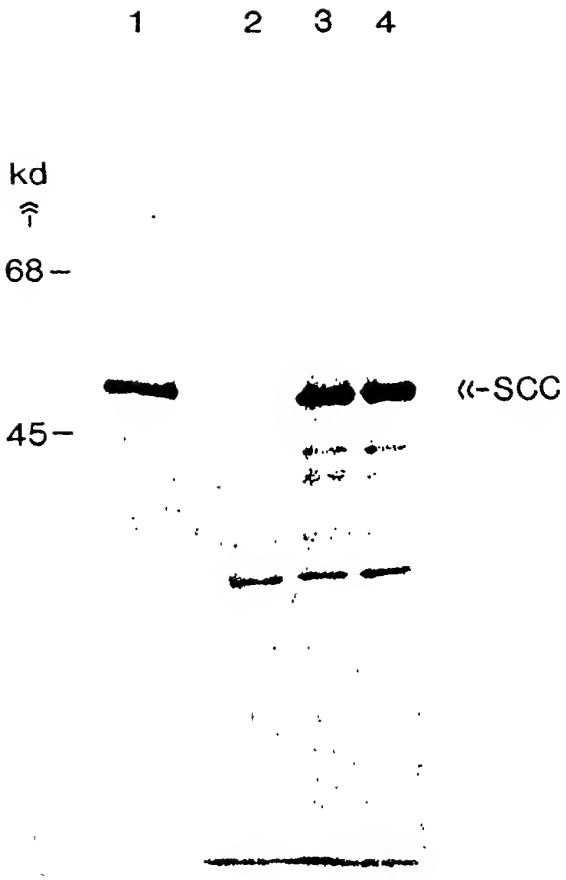
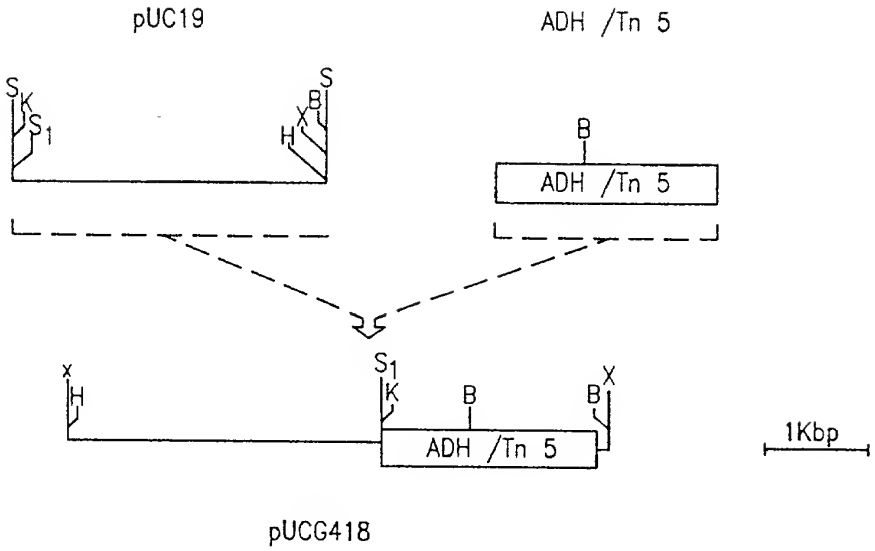
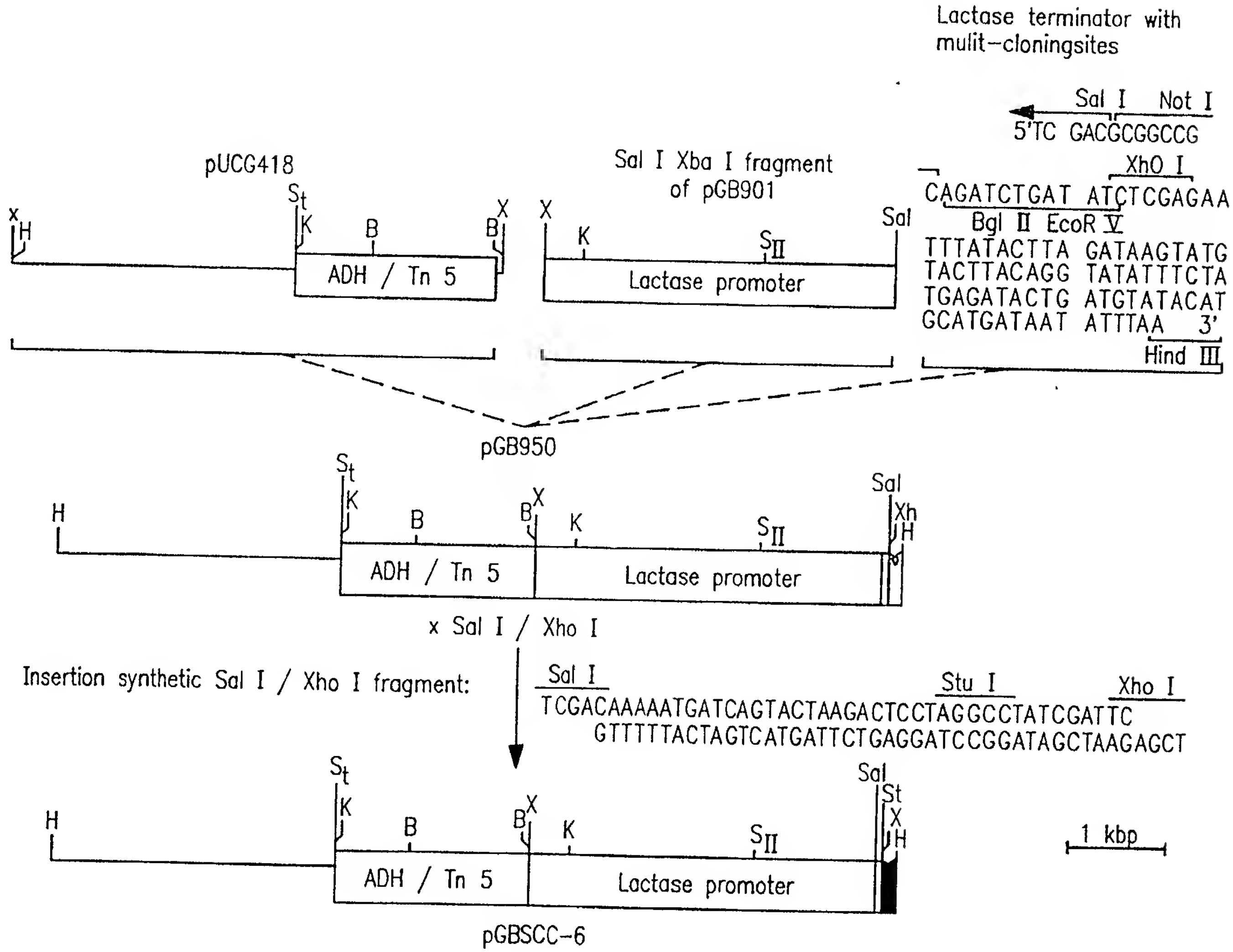


FIG. IIB



pUCG418

FIG. 12



Lactase terminator with
mulit-cloningsites
Sal I
Not I
5'TC GACGCGGCCG
XhO I
CAGATCTGAT ATCTCGAGAA
Bgl II EcoR V
TTTATACTTA GATAAGTATG
TACTTACAGG TATATTTCTA
TGAGATACTG ATGTATACAT
GCATGATAAT ATTTAA 3'
Hind III
pUCG418
Sal I Xba I fragment
of pGB901
ADH / Tn 5
Lactase promoter
pGB950
x Sal I / Xho I
Insertion synthetic Sal I / Xho I fragment:
Sal I
Stu I
Xho I
TCGACAAAAATGATCAGTACTAAGACTCCTAGGCCTATCGATTC
GTTTTTACTAGTCATGATTCTGAGGATCCGGATAGCTAAGAGCT
pGBSCC-6
1 kbp

FIG. 13

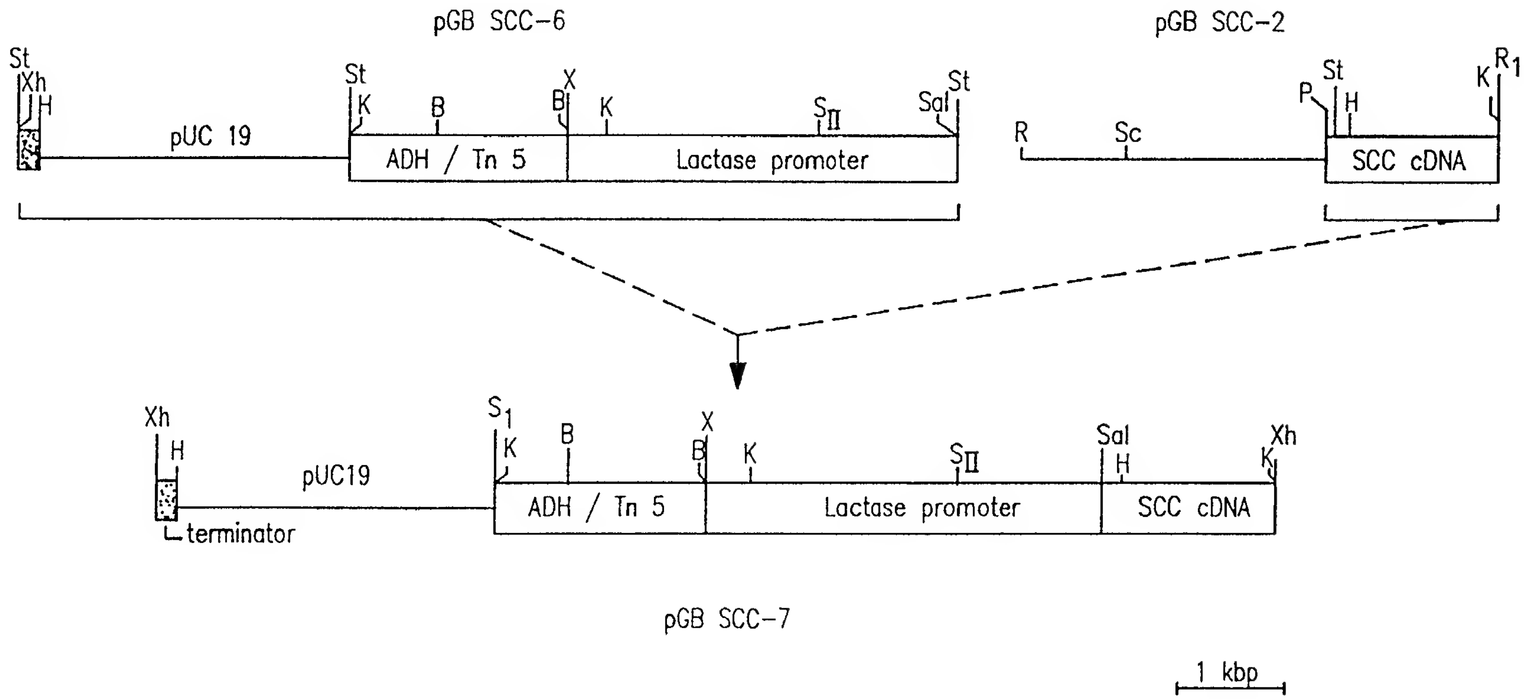

pGB SCC-6
pGB SCC-2
St
Xh
H
pUC 19
St
K
B
X
B
K
S$_{II}$
Sal
St
ADH / Tn 5
Lactase promoter
R
Sc
P
St
H
K
R$_1$
SCC cDNA
Xh
H
pUC19
terminator
S$_1$
K
B
X
B
K
S$_{II}$
Sal
H
Xh
K
ADH / Tn 5
Lactase promoter
SCC cDNA
pGB SCC-7
1 kbp

FIG. 14

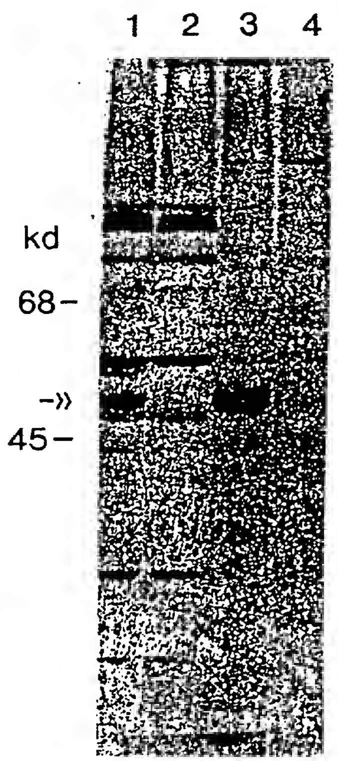


FIG. 15A

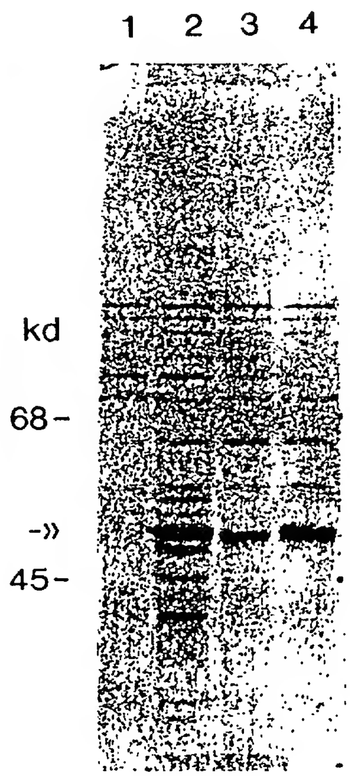


FIG. 15B

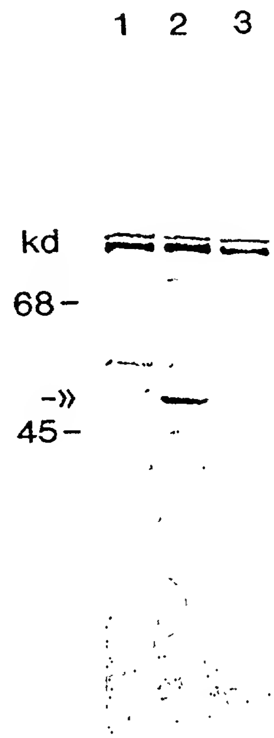


FIG. 15C

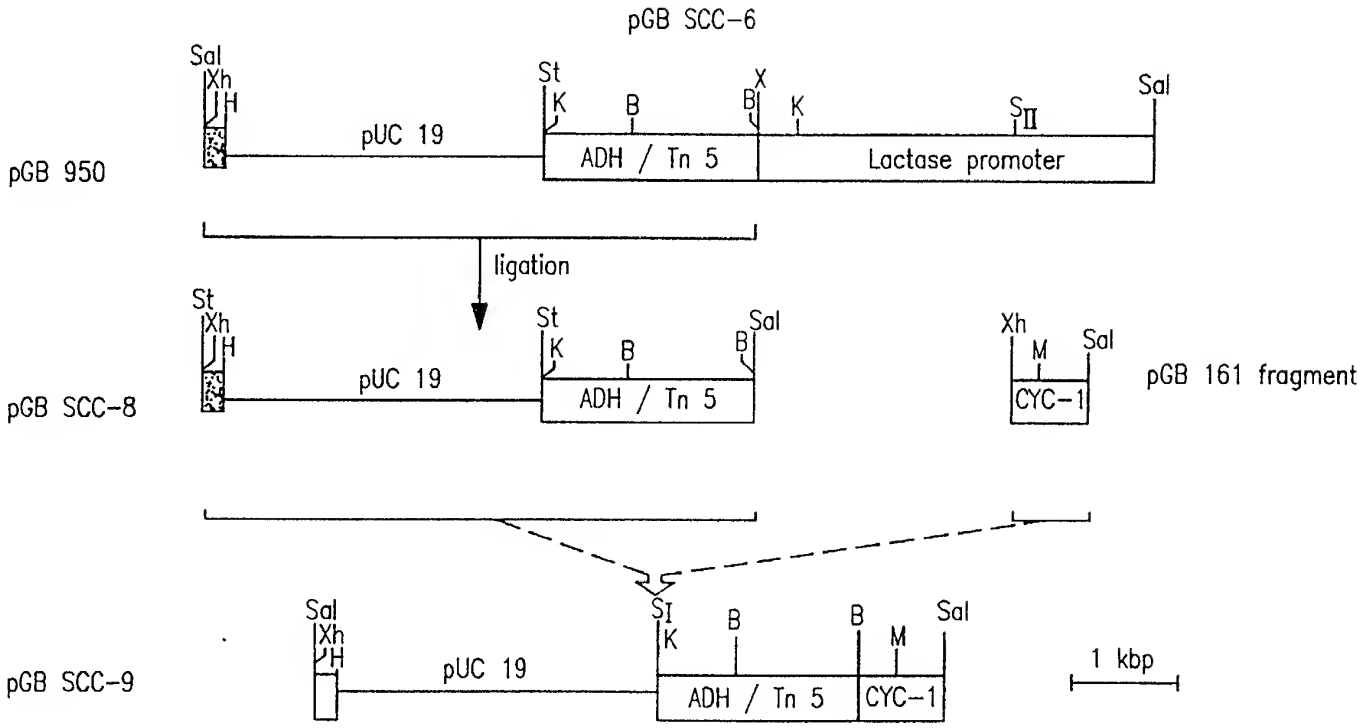


FIG. 16

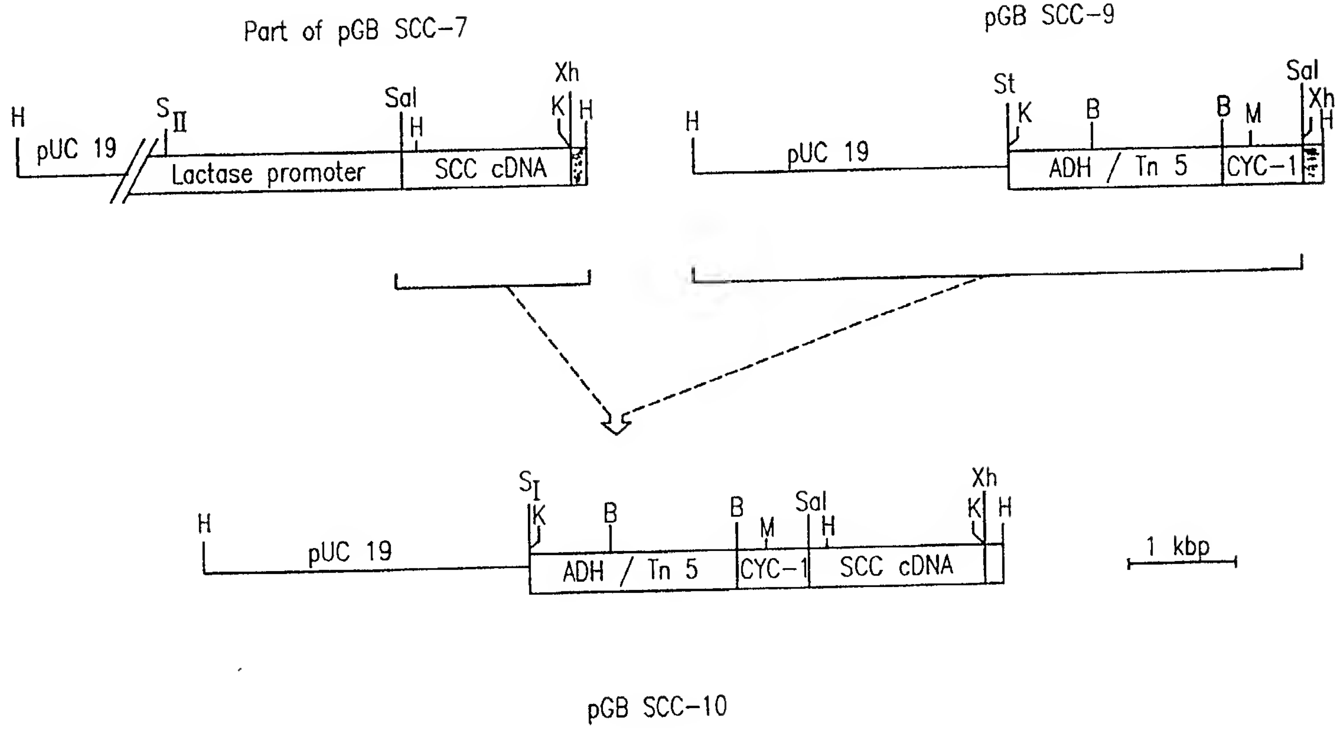

Part of pGB SCC-7
H
pUC 19
SII
Lactase promoter
Sal
H
SCC cDNA
Xh
K
H
pGB SCC-9
H
pUC 19
St
K
B
ADH / Tn 5
B
M
CYC-1
Sal
Xh
H
H
pUC 19
SI
K
B
ADH / Tn 5
B
M
CYC-1
Sal
H
SCC cDNA
Xh
K
H
1 kbp
pGB SCC-10

FIG. 17

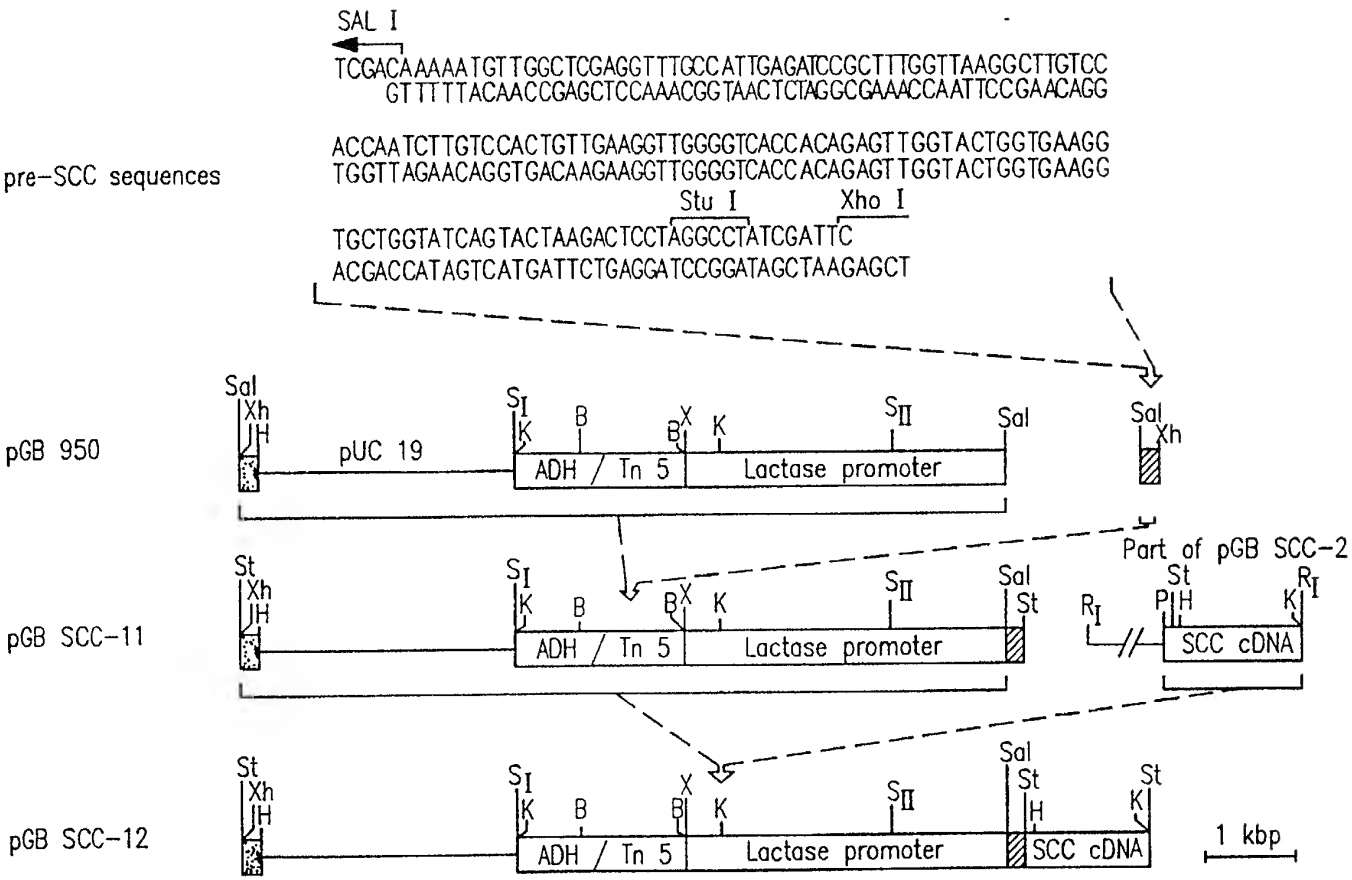
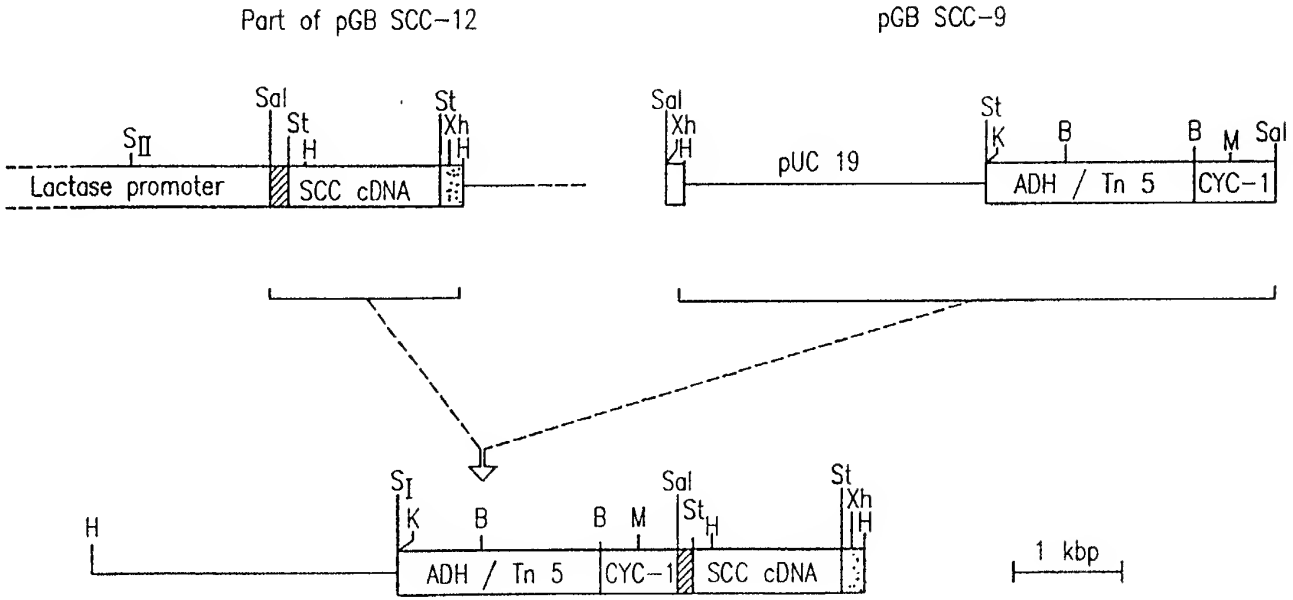


FIG. 18



pGB SCC-13

FIG. 19

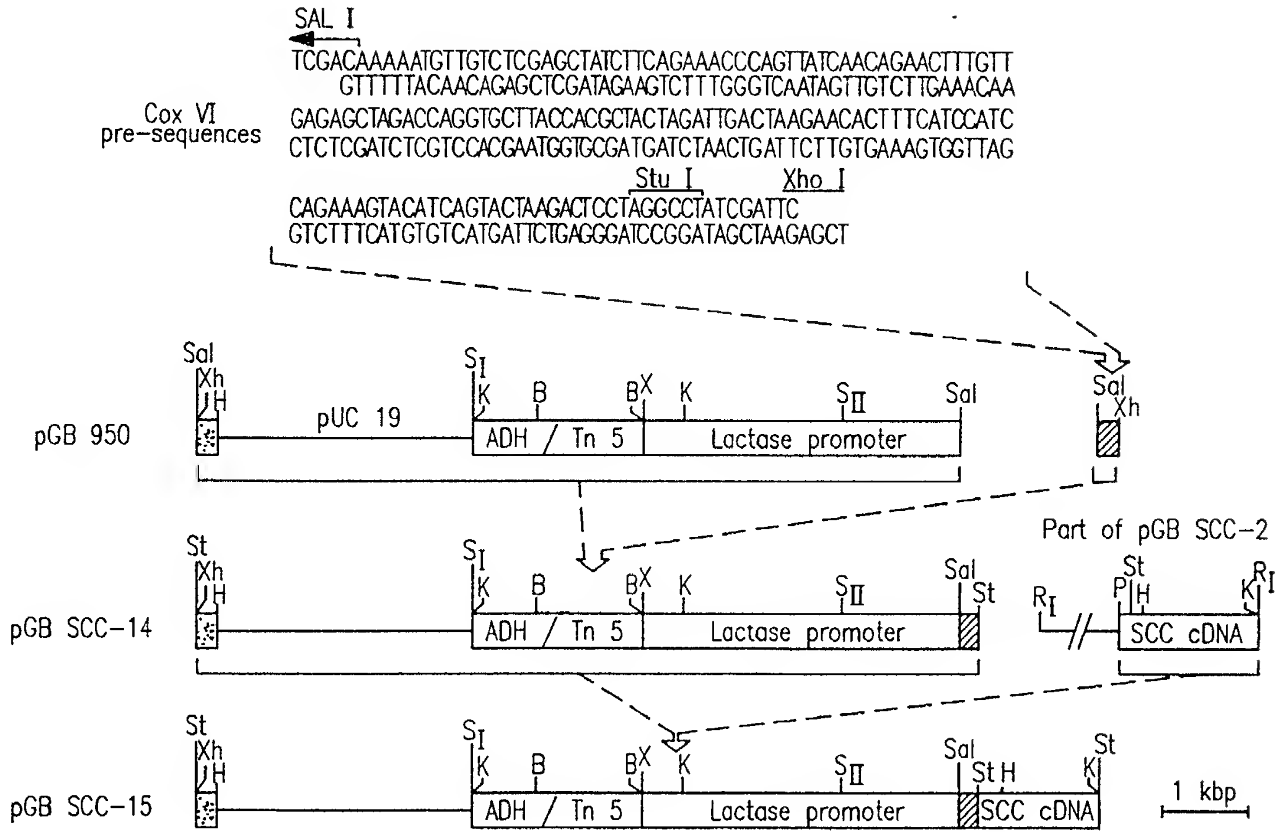

SAL I
TCGACAAAAATGTTGTCTCGAGCTATCTTCAGAAACCCAGTTATCAACAGAACTTTGTT
GTTTTTACAACAGAGCTCGATAGAAGTCTTTGGGTCAATAGTTGTCTTGAAACAA
Cox VI
pre-sequences
GAGAGCTAGACCAGGTGCTTACCACGCTACTAGATTGACTAAGAACACTTTCATCCATC
CTCTCGATCTCGTCCACGAATGGTGCGATGATCTAACTGATTCTTGTGAAAGTGGTTAG
Stu I
Xho I
CAGAAAGTACATCAGTACTAAGACTCCTAGGCCTATCGATTC
GTCTTTCATGTGTCATGATTCTGAGGGATCCGGATAGCTAAGAGCT
pGB 950
pUC 19
ADH / Tn 5
Lactase promoter
pGB SCC-14
Part of pGB SCC-2
SCC cDNA
pGB SCC-15
1 kbp

FIG. 20

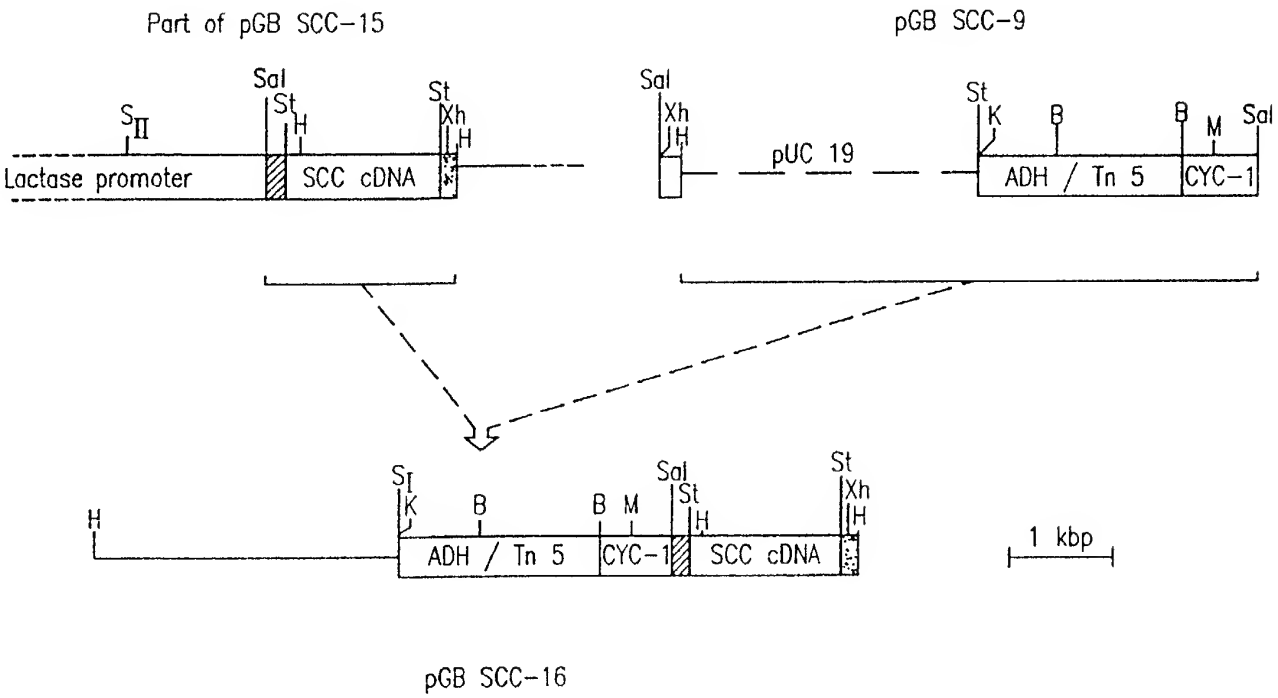


FIG. 21

FIG. 22A

FIG. 22B

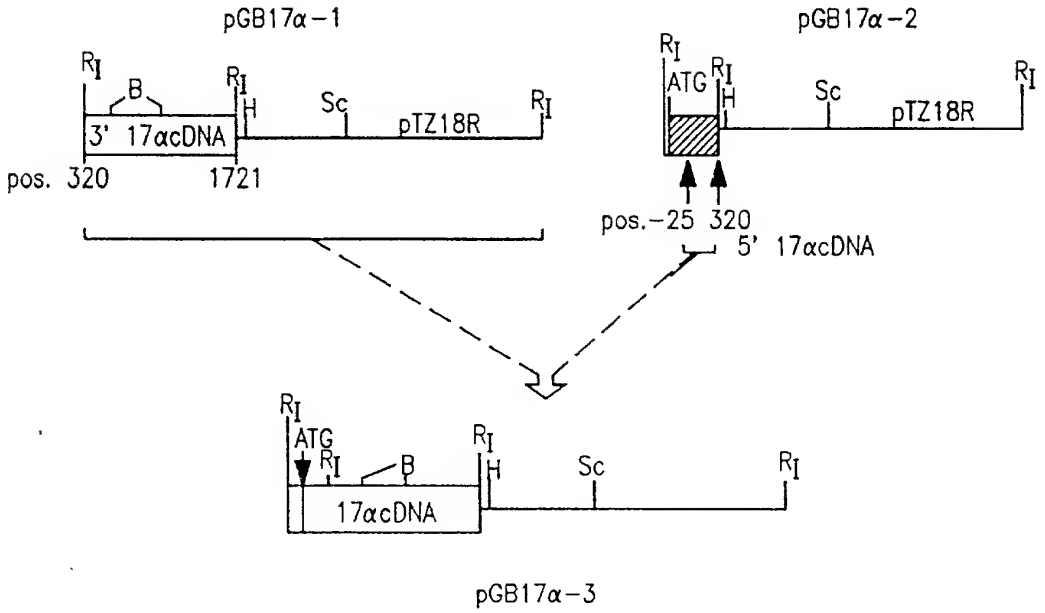


FIG. 22C

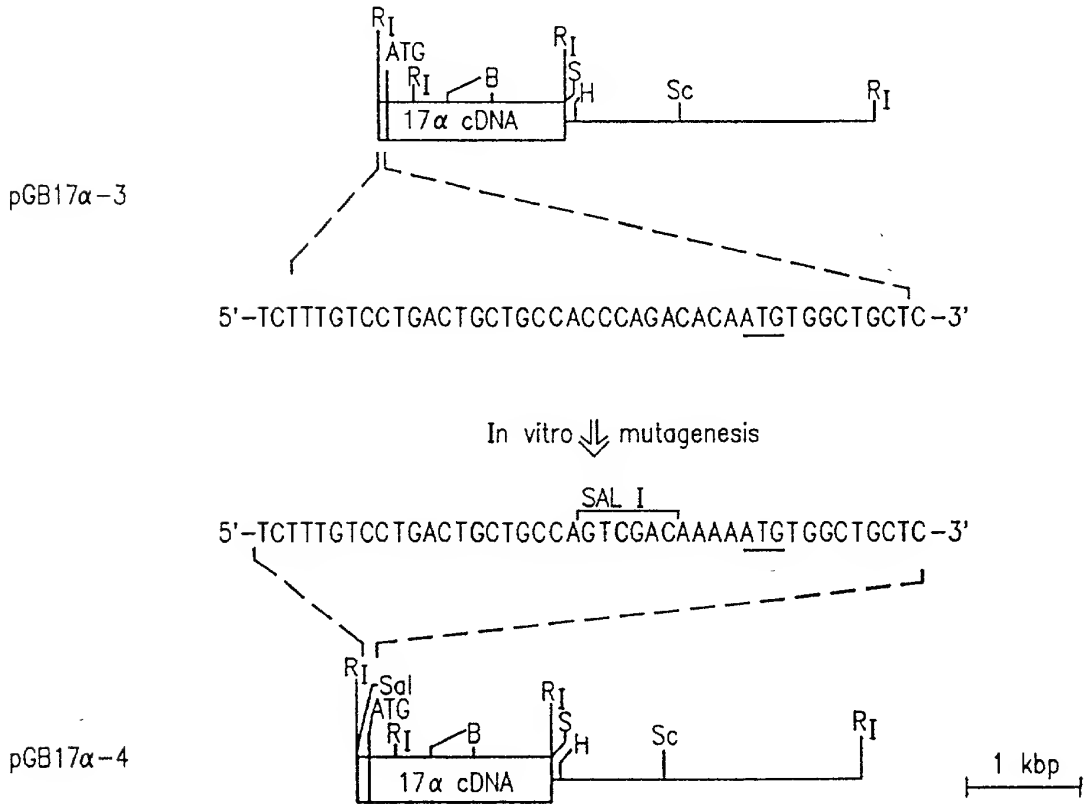
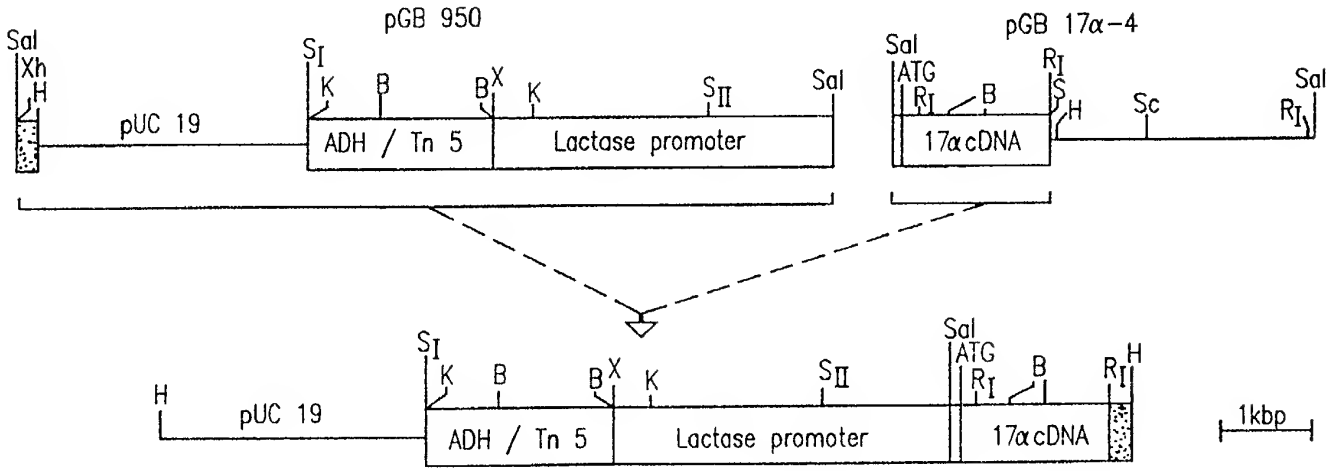


FIG. 23



pGB 17α-5

FIG. 24

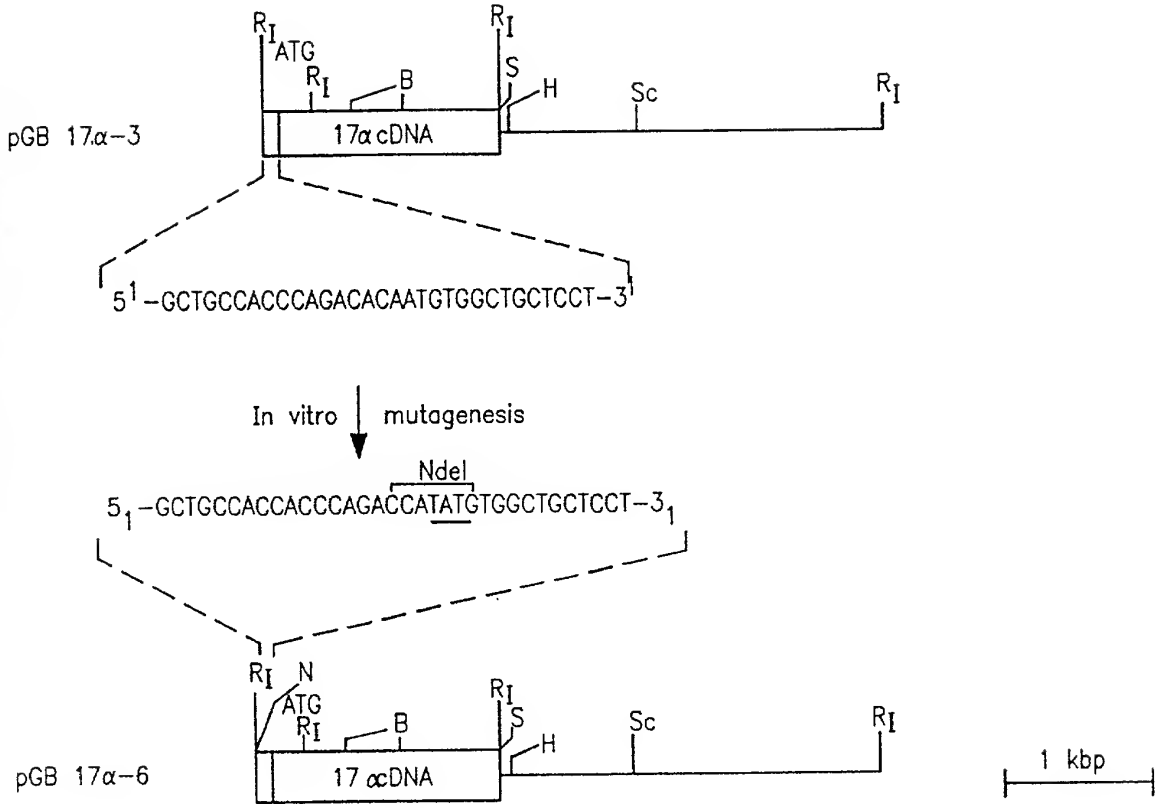


FIG. 25

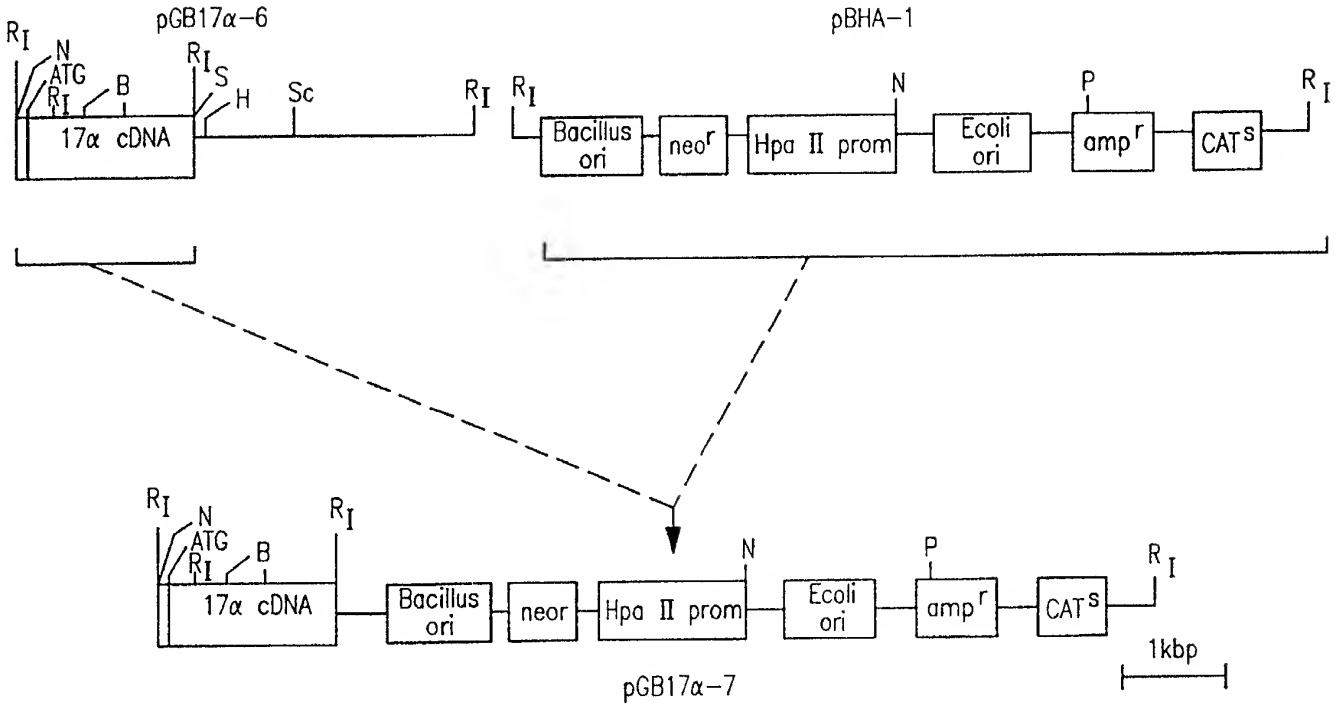

pGB17α-6
pBHA-1
RI
N
ATG
RI
B
RI
S
H
Sc
RI
17α cDNA
RI
Bacillus ori
neor
Hpa II prom
N
Ecoli ori
P
ampr
CATs
RI
RI
N
ATG
RI
B
RI
17α cDNA
Bacillus ori
neor
Hpa II prom
N
Ecoli ori
P
ampr
CATs
RI
1kbp
pGB17α-7

FIG. 26

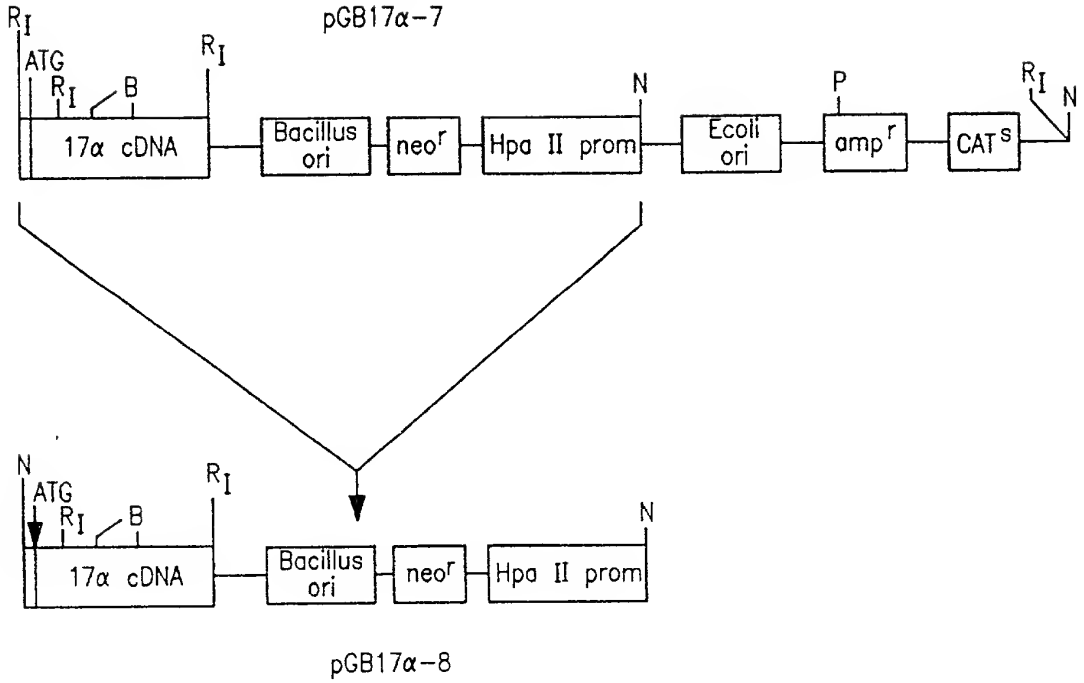

pGB17α-7
ATG
B
17α cDNA
Bacillus ori
neo^r
Hpa II prom
Ecoli ori
amp^r
CAT^s
N
17α cDNA
Bacillus ori
neo^r
Hpa II prom
pGB17α-8

FIG. 27

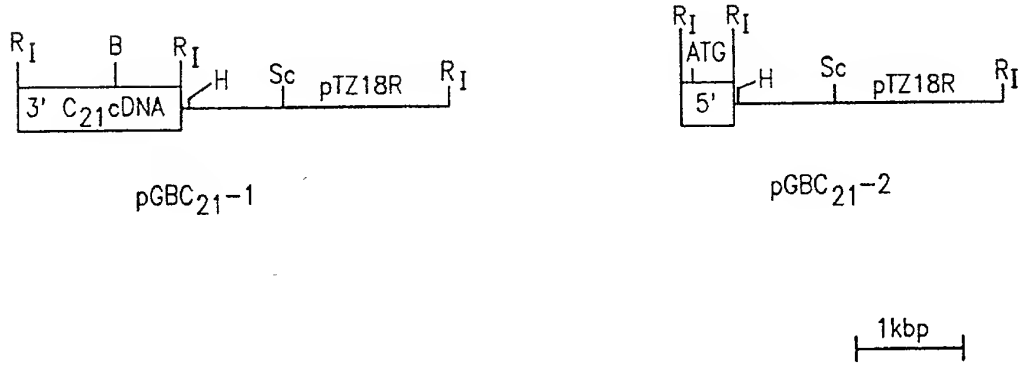

B
3' C21cDNA
H
Sc
pTZ18R
ATG
5'
pGBC21-1
pGBC21-2
1kbp

FIG. 28

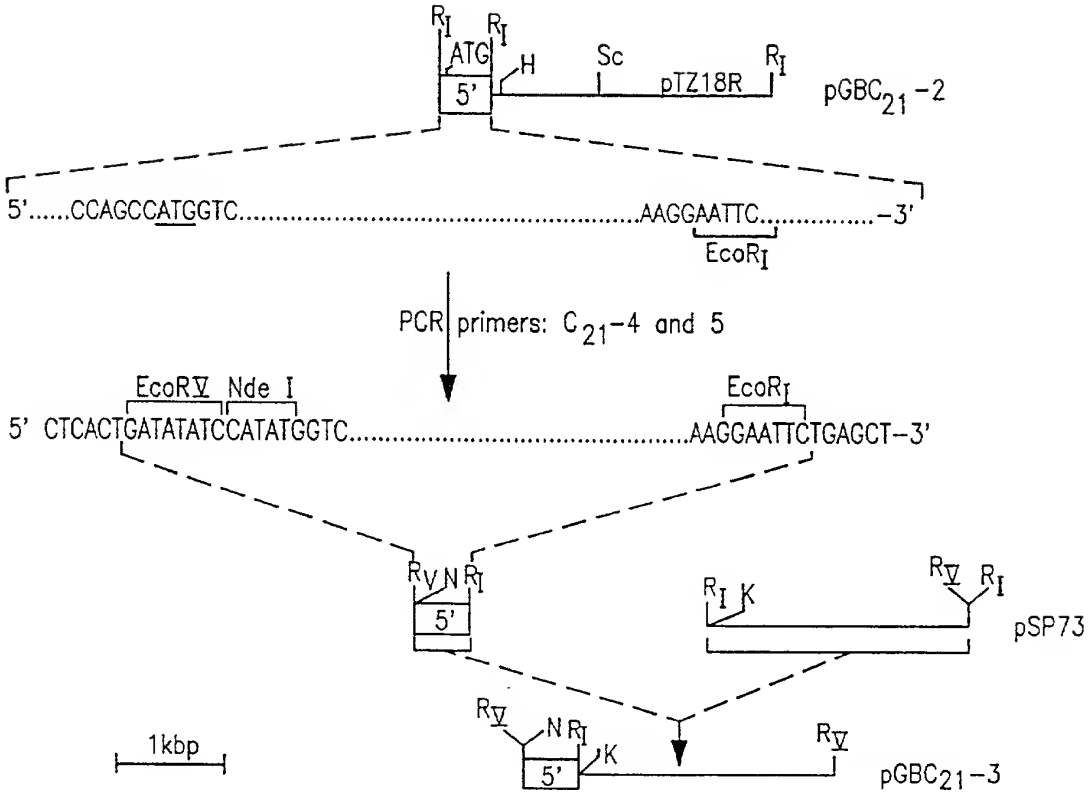

ATG
H
Sc
pTZ18R
5'
pGBC21-2
5'.....CCAGCCATGGTC.....AAGGAATTC.....-3'
EcoRI
PCR primers: C21-4 and 5
EcoRV Nde I
EcoRI
5' CTCACTGATATATCCATATGGTC.....AAGGAATTCTGAGCT-3'
pSP73
1kbp
pGBC21-3

FIG. 29

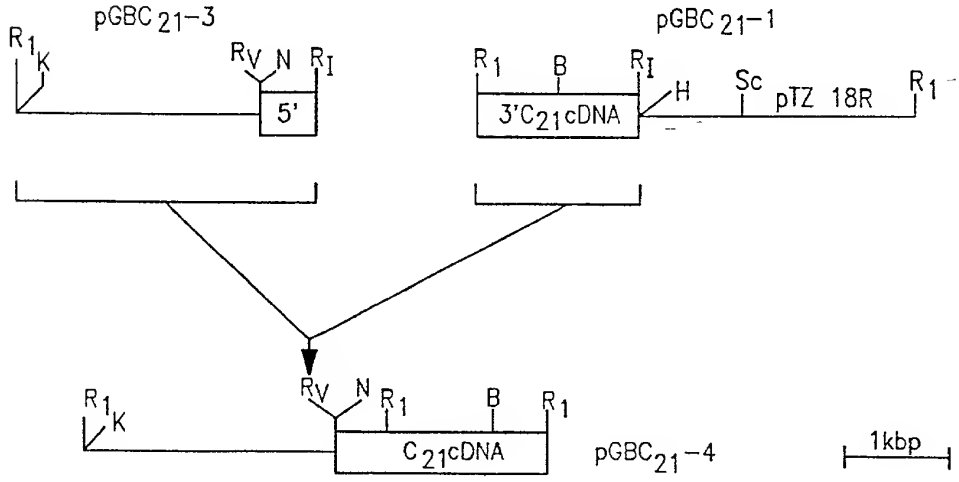

pGBC21-3
pGBC21-1
5'
3'C21cDNA
H
Sc
pTZ 18R
C21cDNA
pGBC21-4
1kbp

FIG. 30

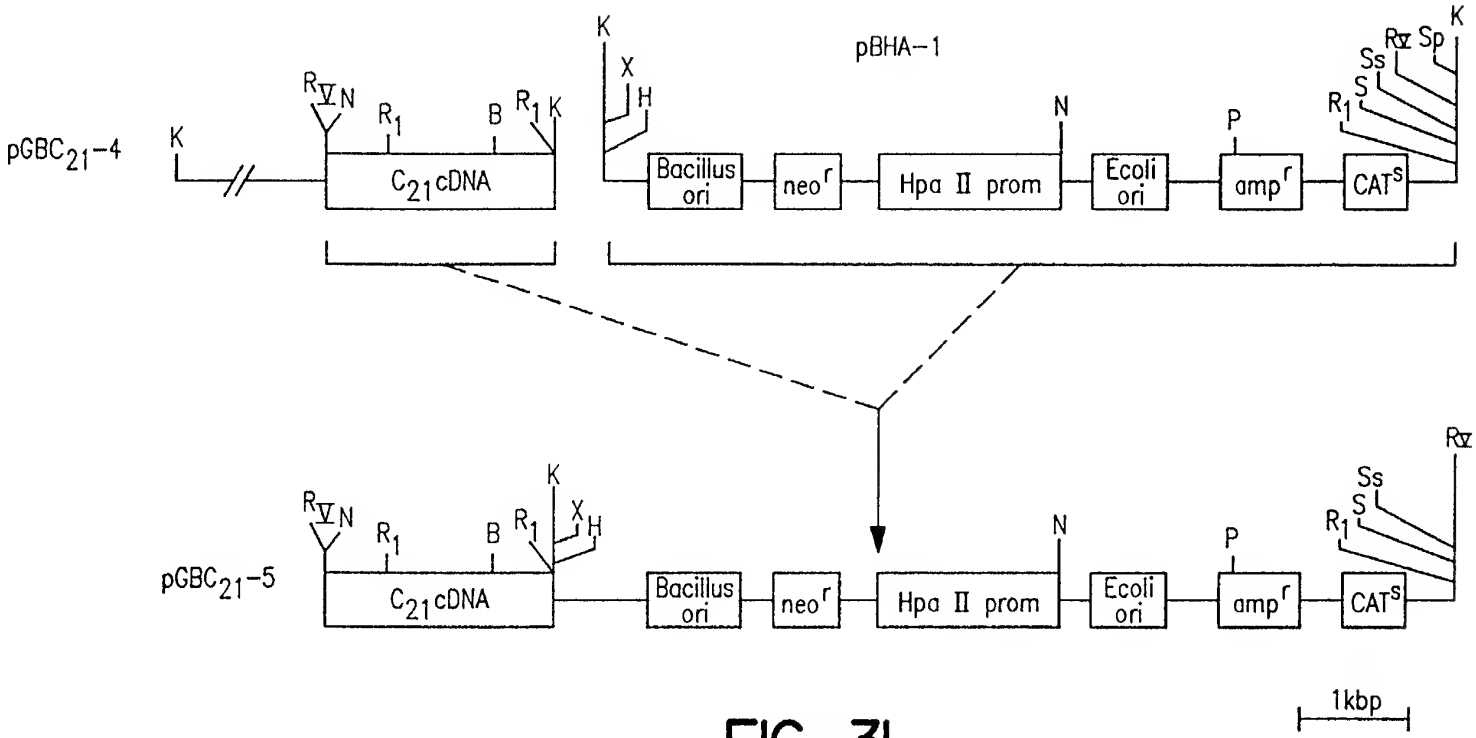

pGBC21-4
K
RV N
R1
B
R1
K
C21 cDNA
pBHA-1
K
X
H
Bacillus ori
neor
N
Hpa II prom
Ecoli ori
P
ampr
R1
S
Ss
RV
Sp
K
CATs
pGBC21-5
K
RV N
R1
B
R1
X
H
C21 cDNA
Bacillus ori
neor
N
Hpa II prom
Ecoli ori
P
ampr
R1
S
Ss
RV
CATs
1kbp

FIG. 31

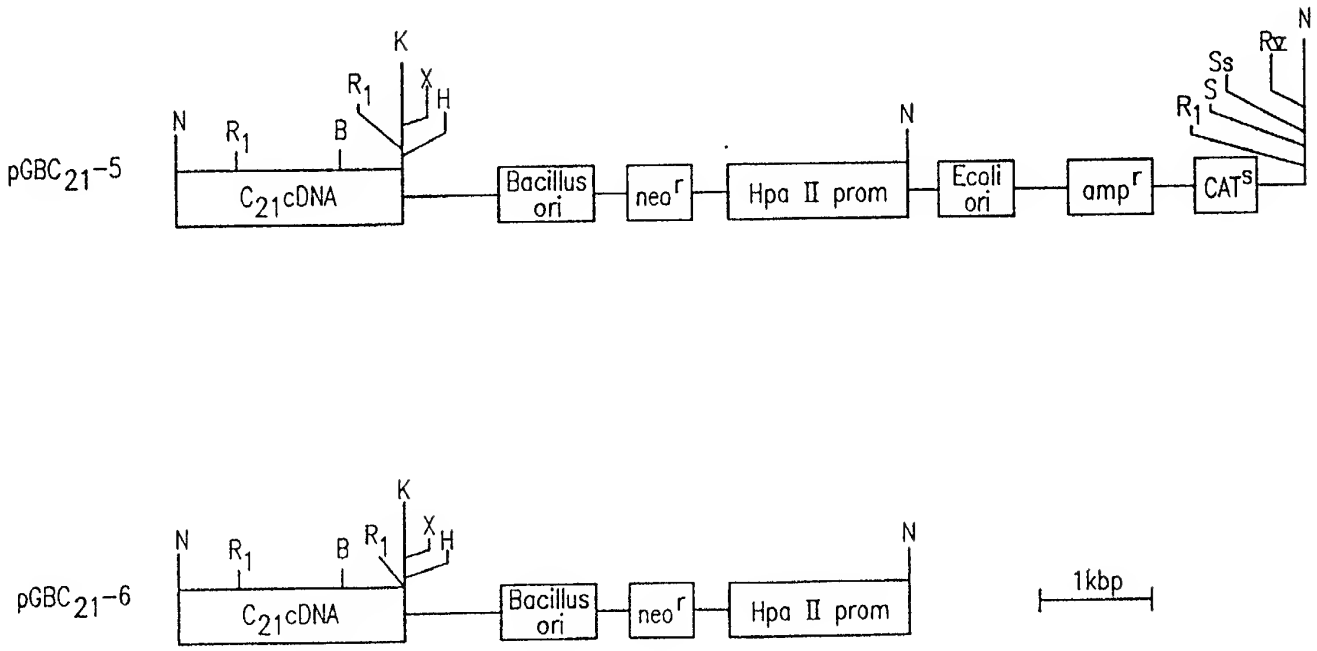

pGBC21-5
N
R1
B
K
R1
X
H
C21cDNA
Bacillus ori
neor
N
Hpa II prom
Ecoli ori
ampr
R1
S
Ss
Rv
N
CATs
pGBC21-6
N
R1
B
K
R1
X
H
C21cDNA
Bacillus ori
neor
N
Hpa II prom
1kbp

FIG. 32

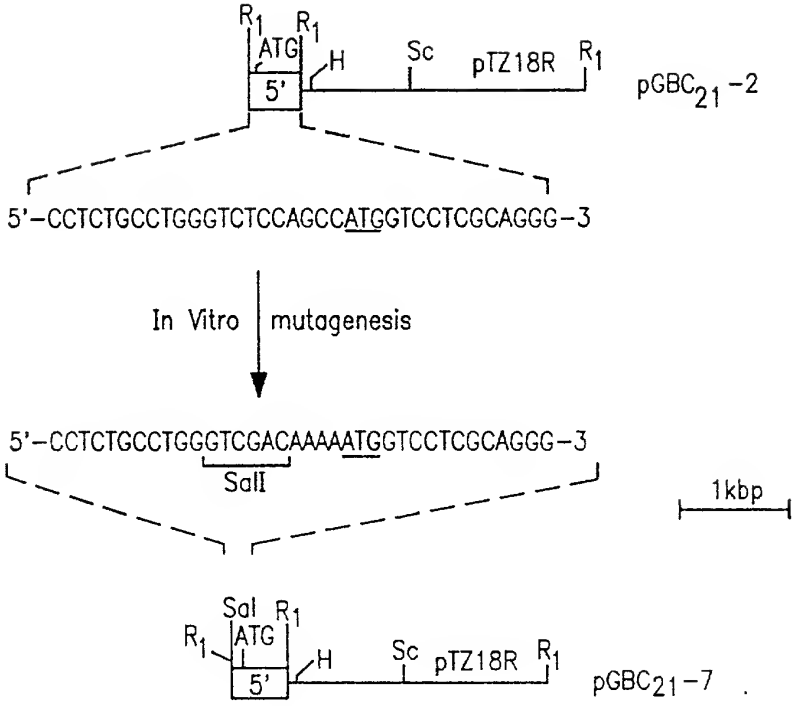

R1 R1
ATG
H
Sc
pTZ18R
R1
5'
pGBC21-2
5'-CCTCTGCCTGGGTCTCCAGCCATGGTCCTCGCAGGG-3
In Vitro mutagenesis
5'-CCTCTGCCTGGGTCGACAAAAATGGTCCTCGCAGGG-3
SalI
1kbp
Sal R1
R1
ATG
H
Sc
pTZ18R
R1
5'
pGBC21-7

FIG. 33

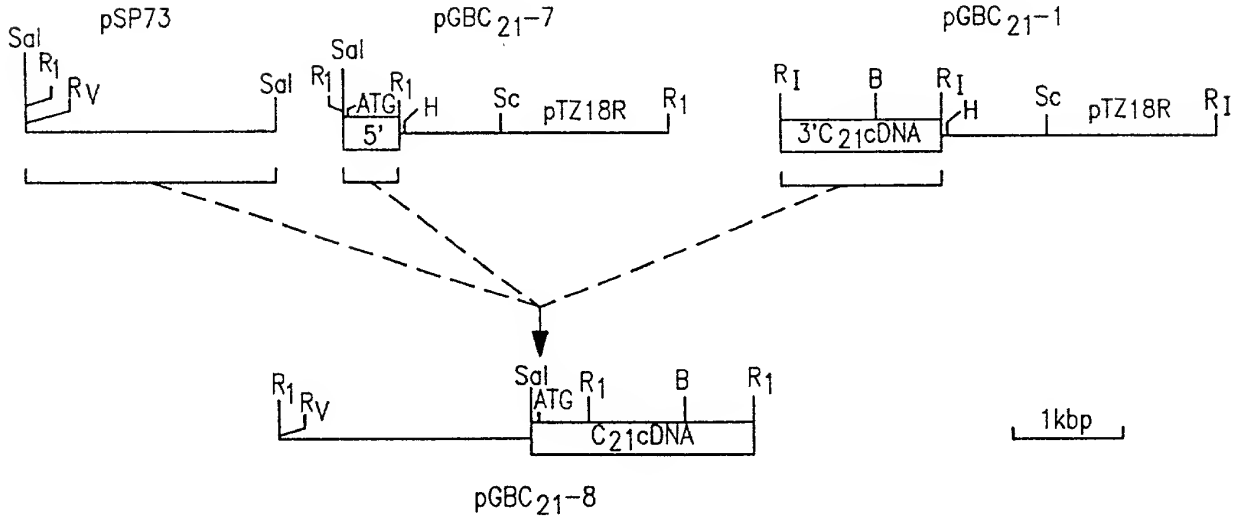

pSP73
Sal
R1
RV
Sal
pGBC21-7
Sal
R1
R1
ATG
H
Sc
pTZ18R
R1
5'
pGBC21-1
RI
B
RI
H
Sc
pTZ18R
RI
3'C21cDNA
R1 RV
Sal
ATG
R1
B
R1
C21cDNA
1kbp
pGBC21-8

FIG. 34

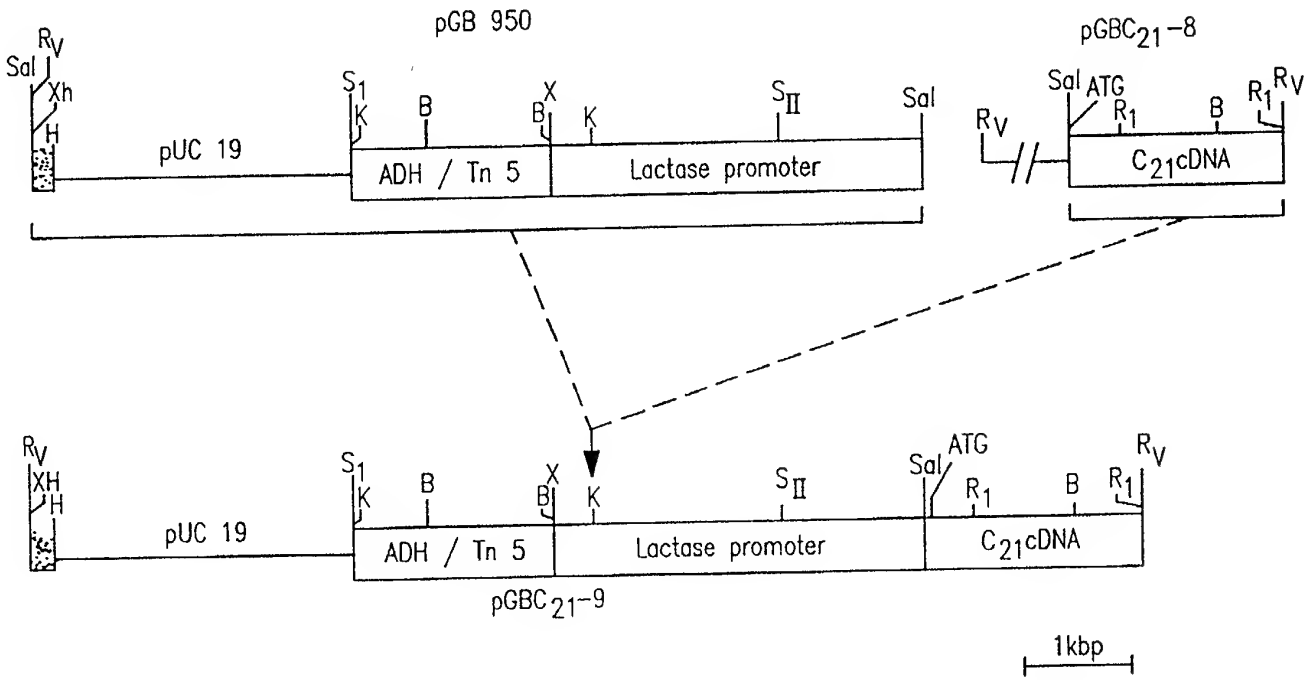


FIG. 35

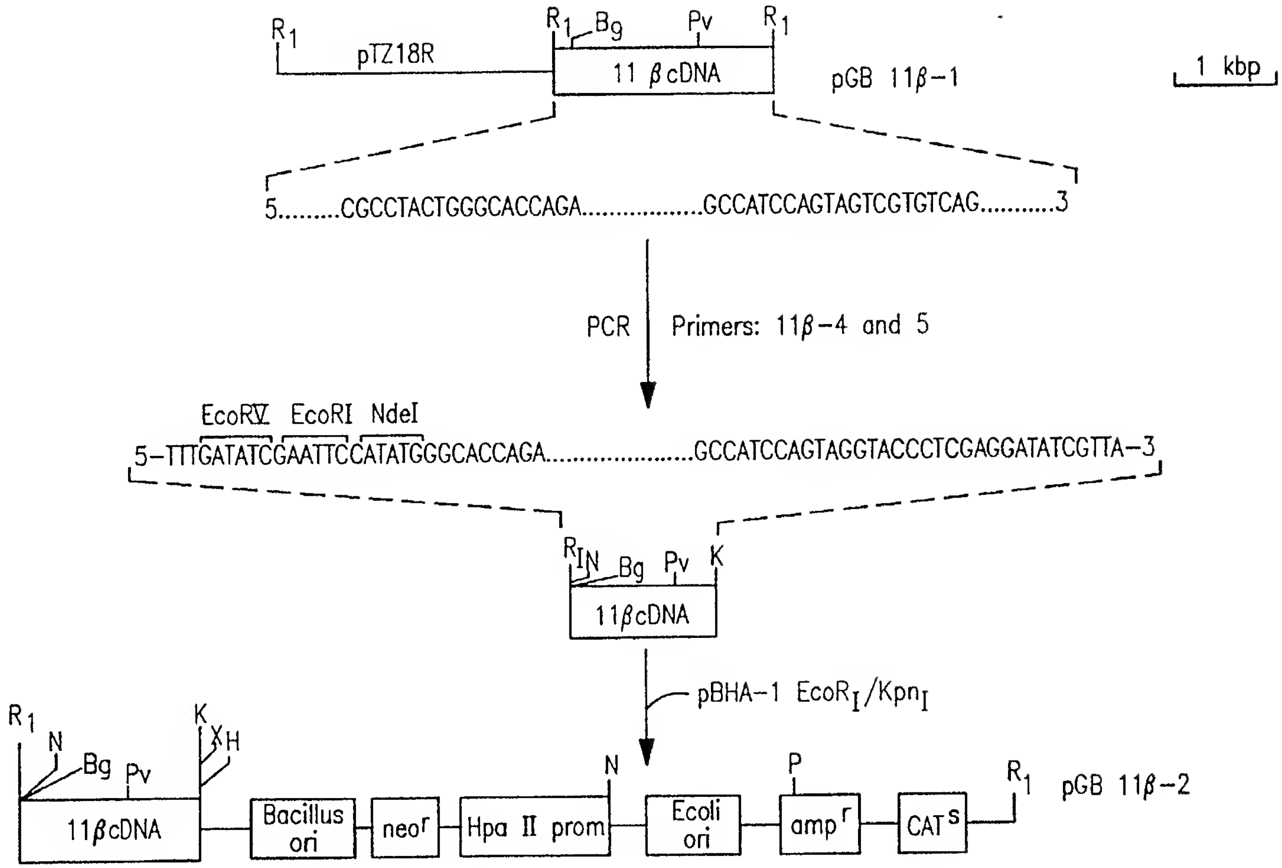

R1
pTZ18R
R1
Bg
Pv
R1
11 βcDNA
pGB 11β-1
1 kbp
5.........CGCCTACTGGGCACCAGA.................GCCATCCAGTAGTCGTGTCAG...........3
PCR
Primers: 11β-4 and 5
EcoRV
EcoRI
NdeI
5-TTTGATATCGAATTCCATATGGGCACCAGA.....................GCCATCCAGTAGGTACCCTCGAGGATATCGTTA-3
RI
N
Bg
Pv
K
11βcDNA
pBHA-1 EcoRI/KpnI
R1
N
Bg
Pv
K
X
H
11βcDNA
Bacillus ori
neor
Hpa II prom
N
Ecoli ori
P
ampr
CATs
R1
pGB 11β-2

FIG. 36

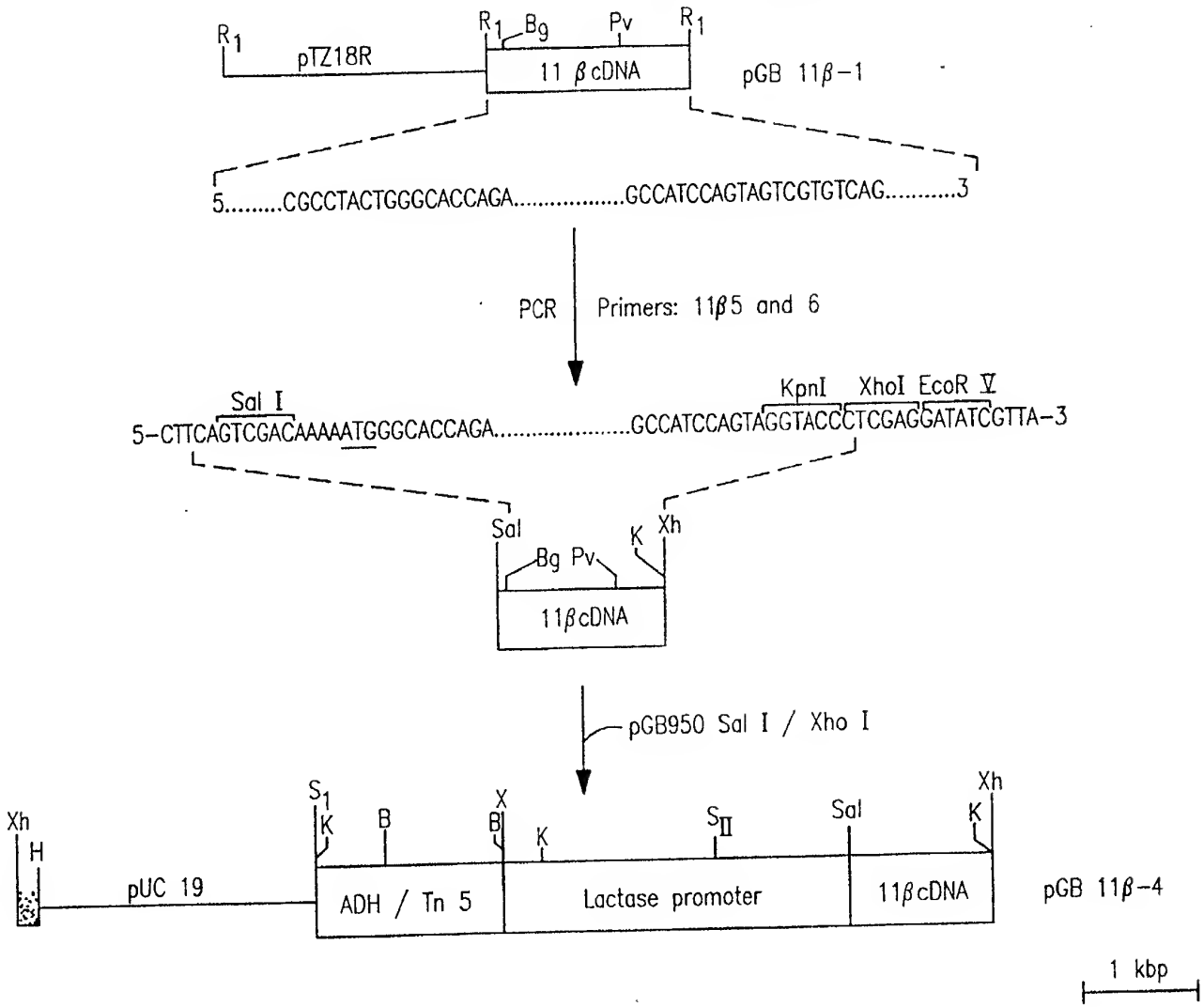

R1
pTZ18R
R1 Bg Pv R1
11 β cDNA
pGB 11β-1
5.....CGCCTACTGGGCACCAGA.....GCCATCCAGTAGTCGTGTCAG.....3
PCR
Primers: 11β5 and 6
Sal I
KpnI XhoI EcoR V
5-CTTCAGTCGACAAAAATGGGCACCAGA.....GCCATCCAGTAGGTACCCTCGAGGATATCGTTA-3
Sal
Bg Pv
K
Xh
11βcDNA
pGB950 Sal I / Xho I
Xh
H
pUC 19
S1
K
B
X
Bl
K
SII
Sal
Xh
K
ADH / Tn 5
Lactase promoter
11βcDNA
pGB 11β-4
1 kbp

FIG. 37

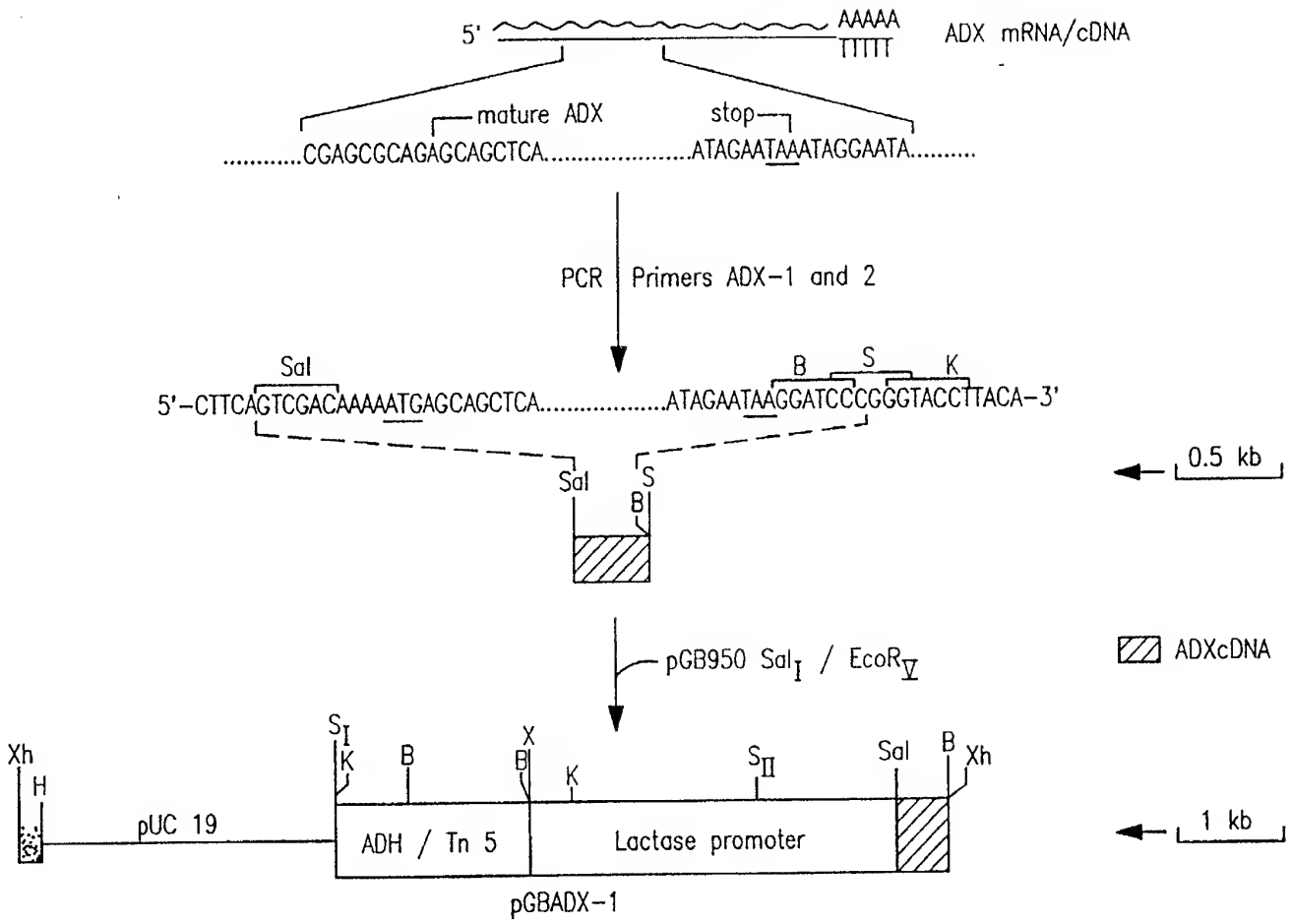

5'
AAAAA
TTTTT
ADX mRNA/cDNA
mature ADX
stop
.....CGAGCGCAGAGCAGCTCA.....ATAGAATAAATAGGAATA.....
PCR
Primers ADX-1 and 2
Sal
B
S
K
5'-CTTCAGTCGACAAAAATGAGCAGCTCA.....ATAGAATAAGGATCCCGGGTACCTTACA-3'
Sal
S
B
0.5 kb
ADXcDNA
pGB950 Sal I / EcoR V
Xh
H
S I
K
B
X
B
K
S II
Sal
B
Xh
pUC 19
ADH / Tn 5
Lactase promoter
1 kb
pGBADX-1

FIG. 38

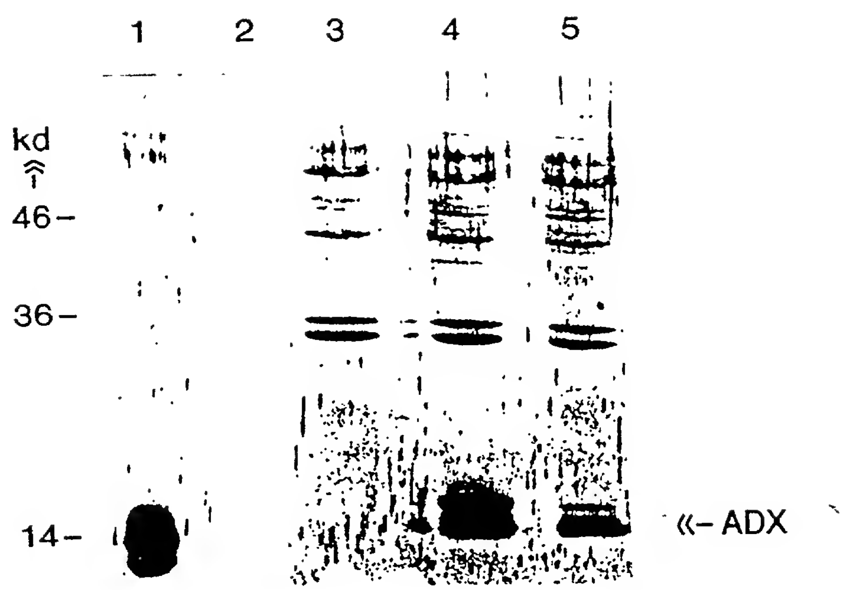


FIG. 39

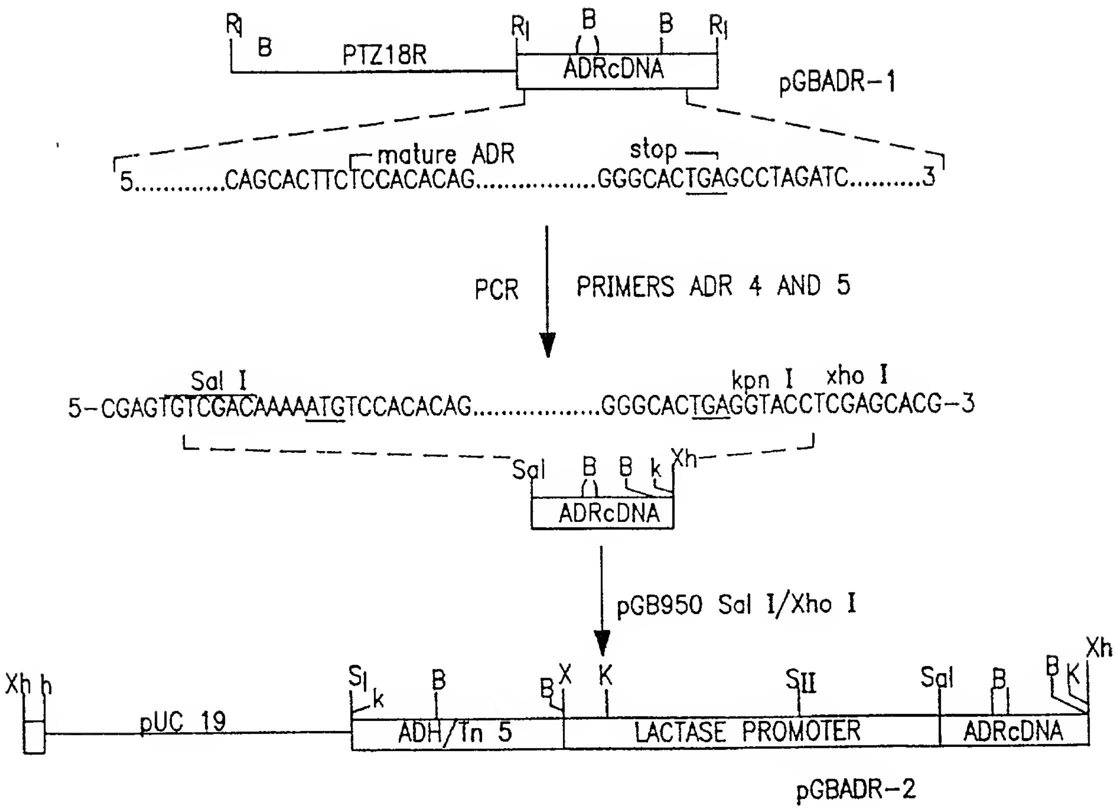
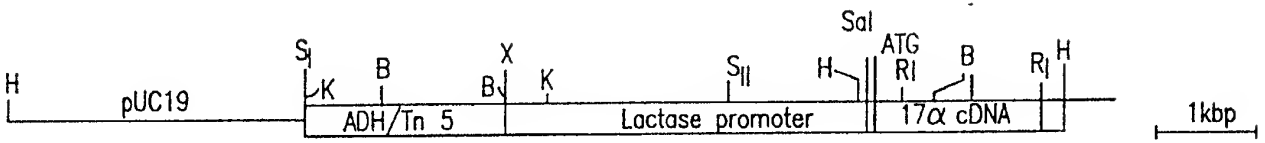

RI
B
PTZ18R
RI
B
B
RI
ADRcDNA
pGBADR-1
mature ADR
stop
5............CAGCACTTCTCCACACAG................GGGCACTGAGCCTAGATC..........3
PCR
PRIMERS ADR 4 AND 5
Sal I
kpn I
xho I
5-CGAGTGTCGACAAAAATGTCCACACAG.................GGGCACTGAGGTACCTCGAGCACG-3
Sal
B
B
k
Xh
ADRcDNA
pGB950 Sal I/Xho I
Xh
h
pUC 19
SI
k
B
B
X
K
SII
Sal
B
B
K
Xh
ADH/Tn 5
LACTASE PROMOTER
ADRcDNA
pGBADR-2

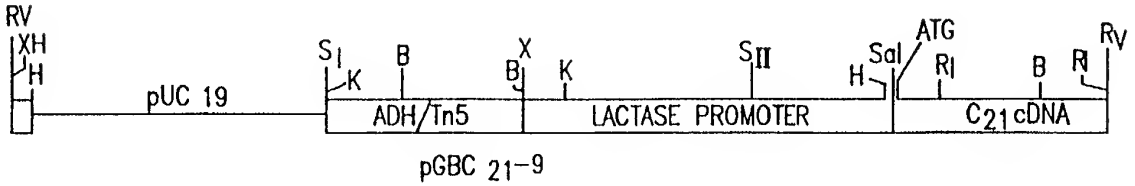
FIG. 40



pGB 17α-5

Lactase terminator

FIG. 41



pGBC 21-9

Lactase terminator

FIG. 42

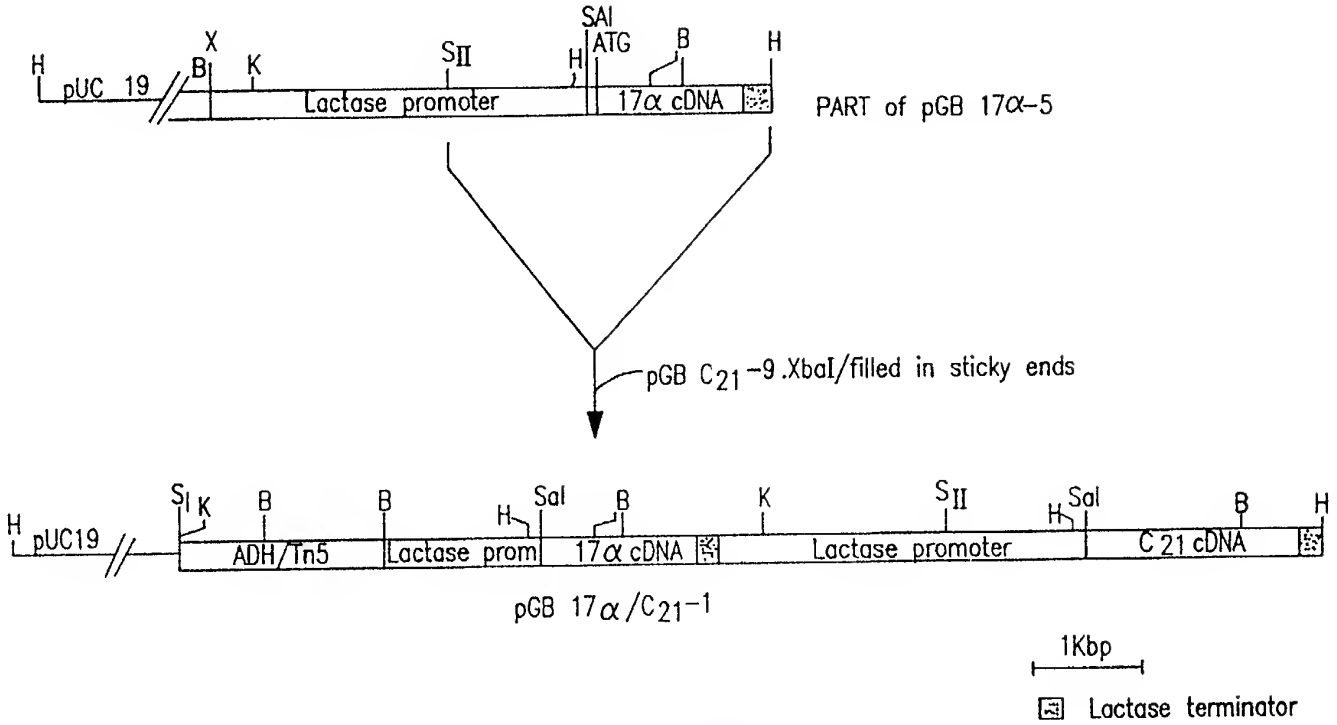


FIG. 43

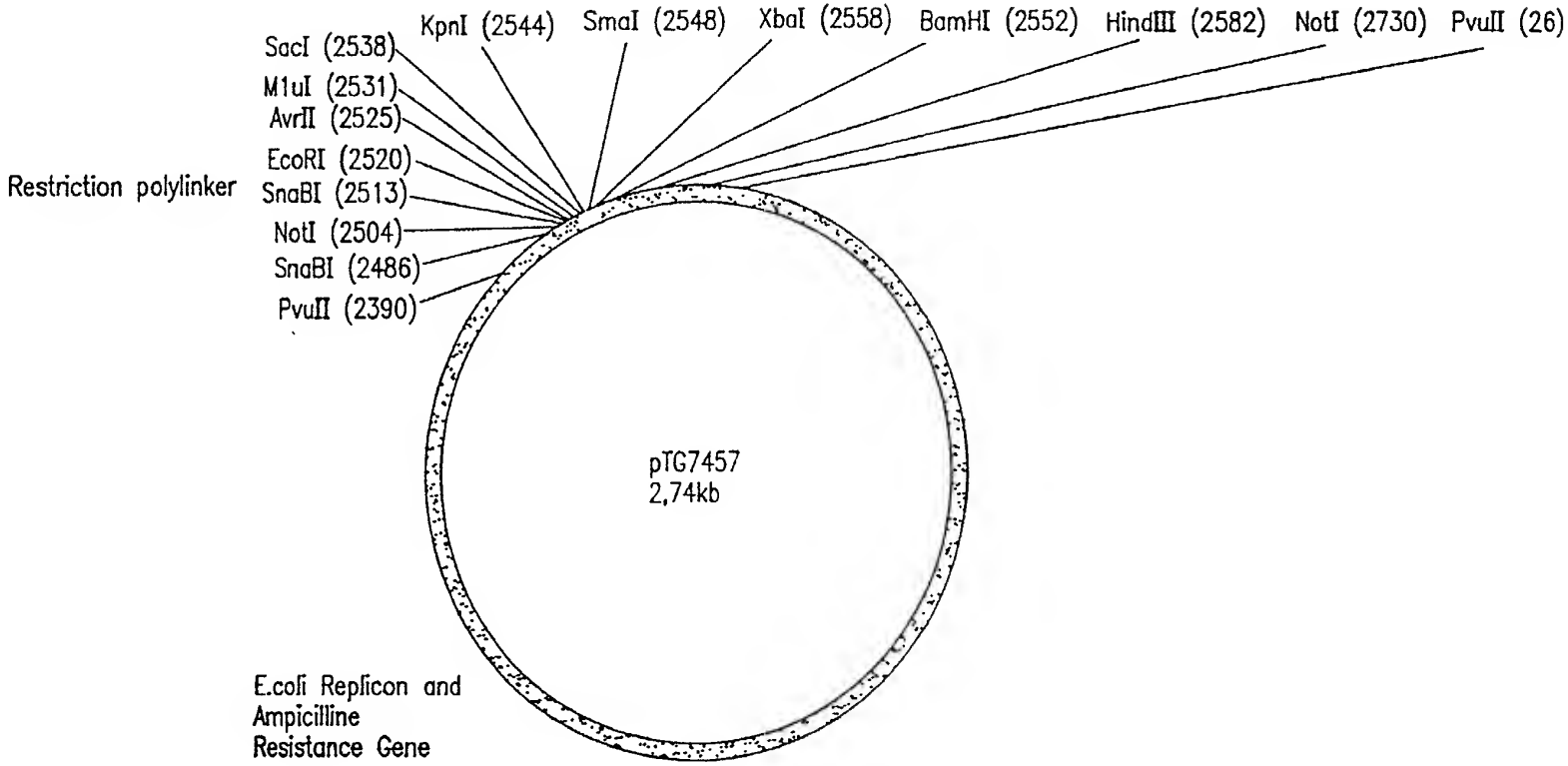

KpnI (2544)
SmaI (2548)
XbaI (2558)
BamHI (2552)
HindIII (2582)
NotI (2730)
PvuII (26)
SacI (2538)
M1uI (2531)
AvrII (2525)
EcoRI (2520)
SnaBI (2513)
NotI (2504)
SnaBI (2486)
PvuII (2390)
Restriction polylinker
pTG7457
2,74kb
E.coli Replicon and
Ampicilline
Resistance Gene

FIG. 44

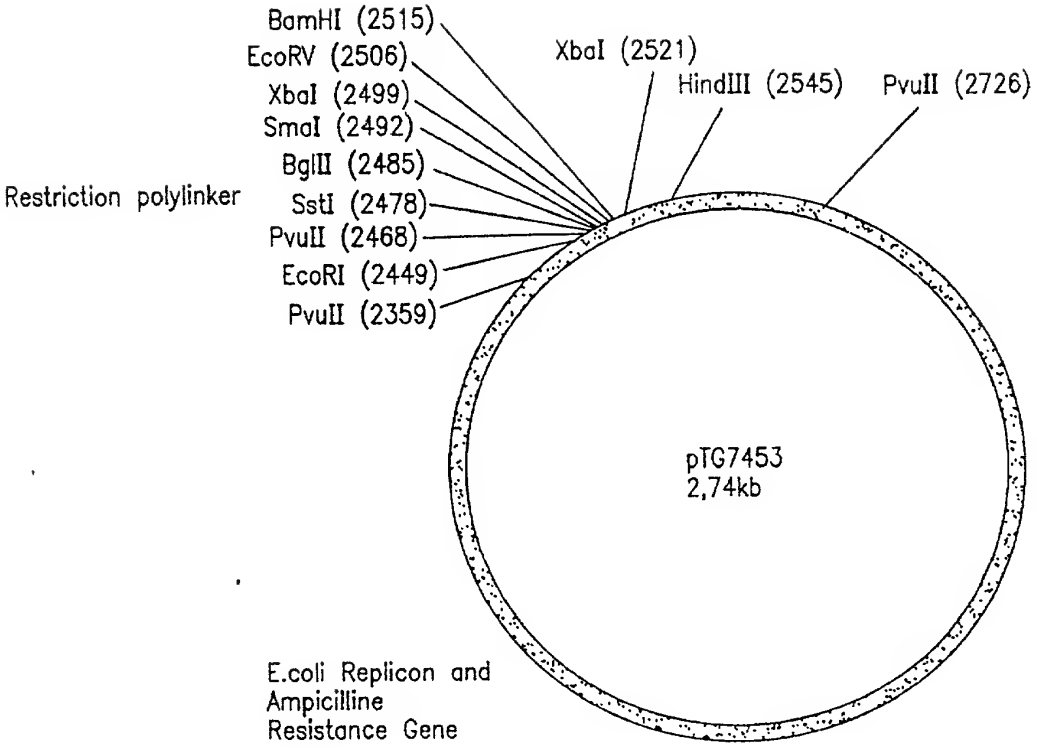


FIG. 45

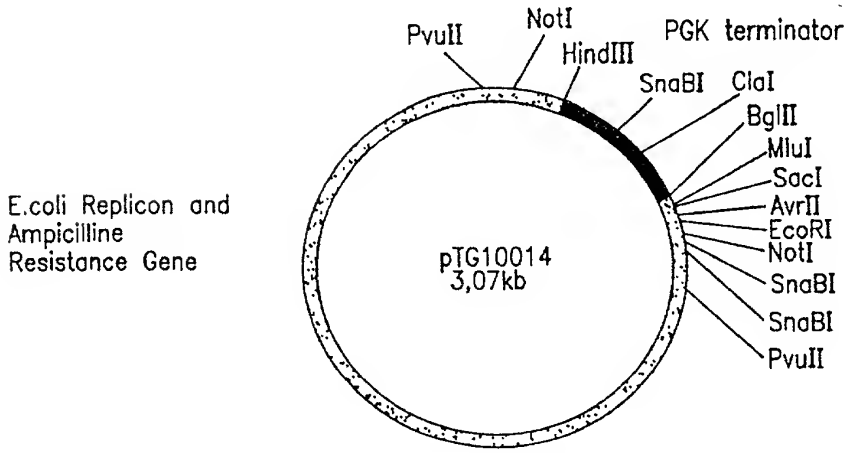


FIG. 46

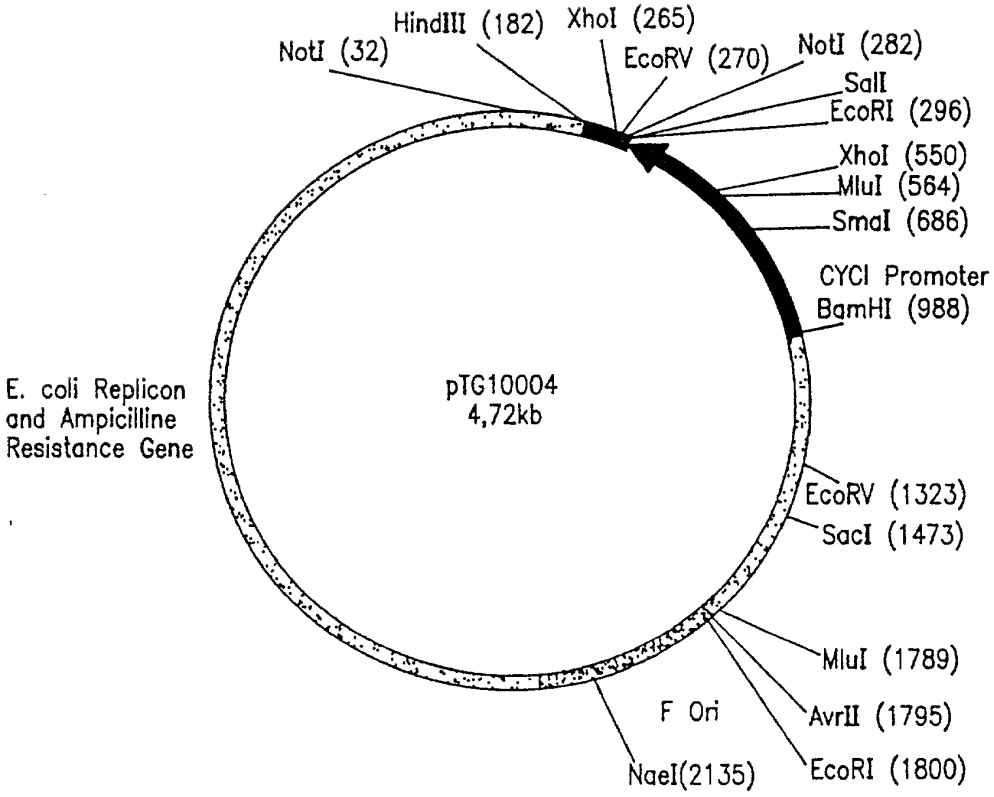


FIG. 47

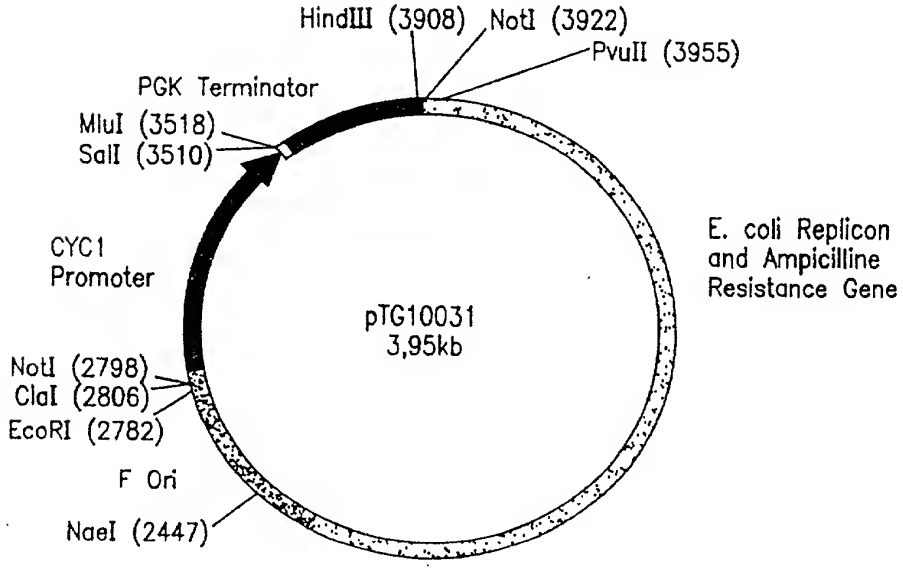


FIG. 48

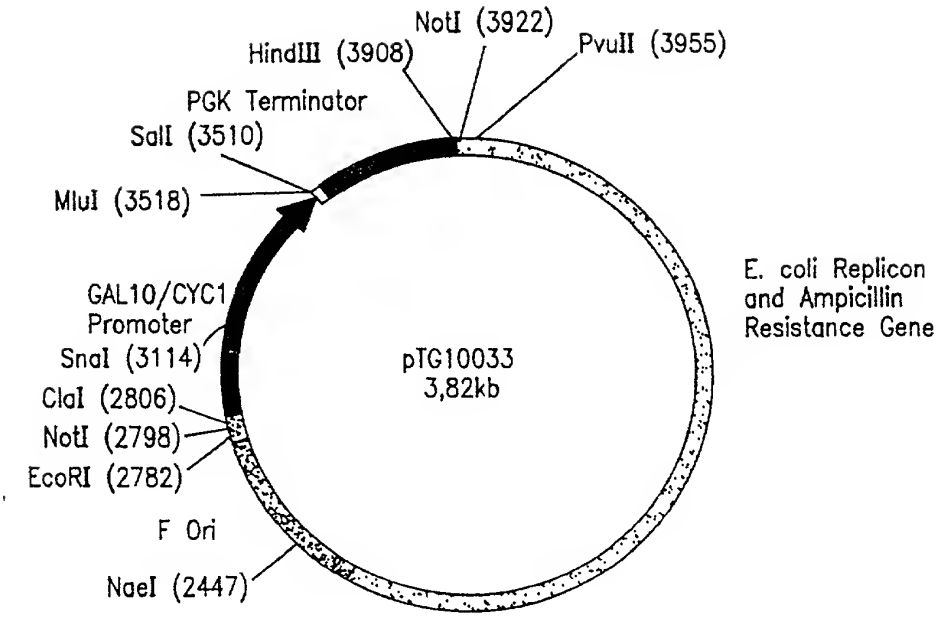


FIG. 49

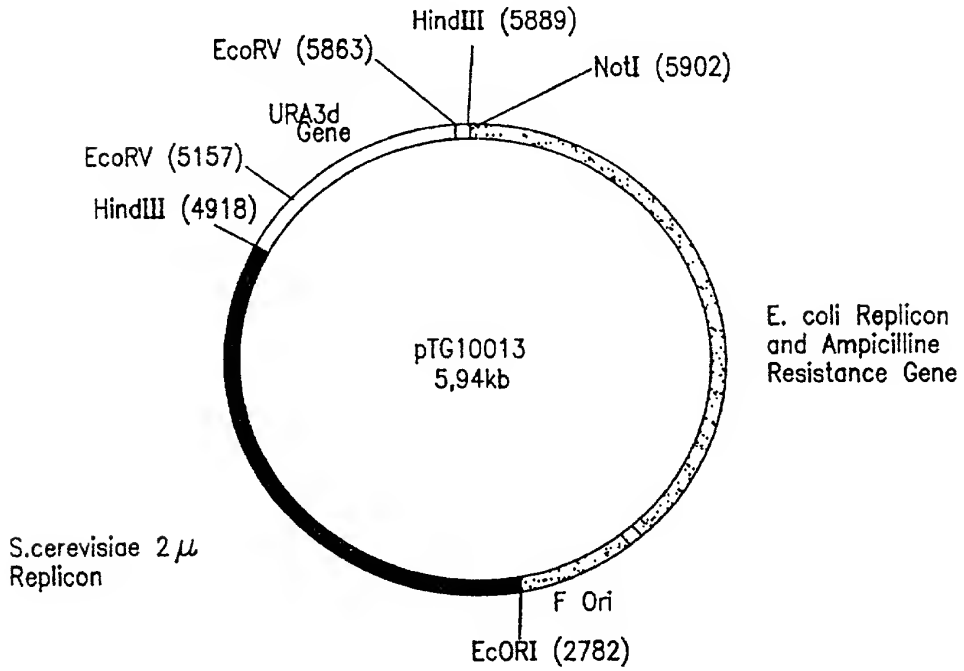


FIG. 50

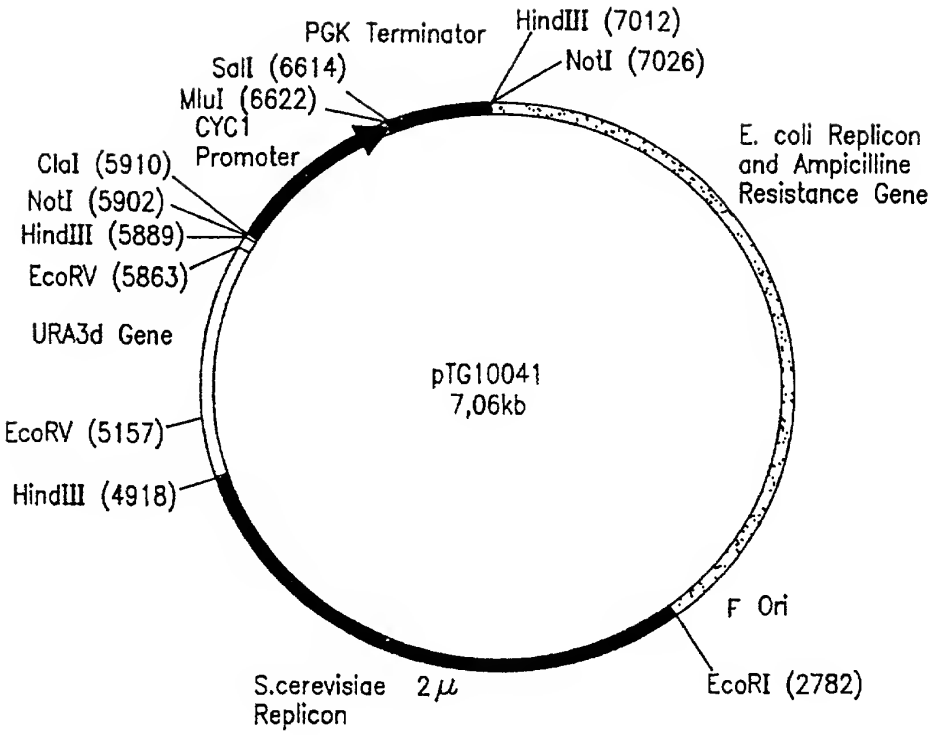


FIG. 51

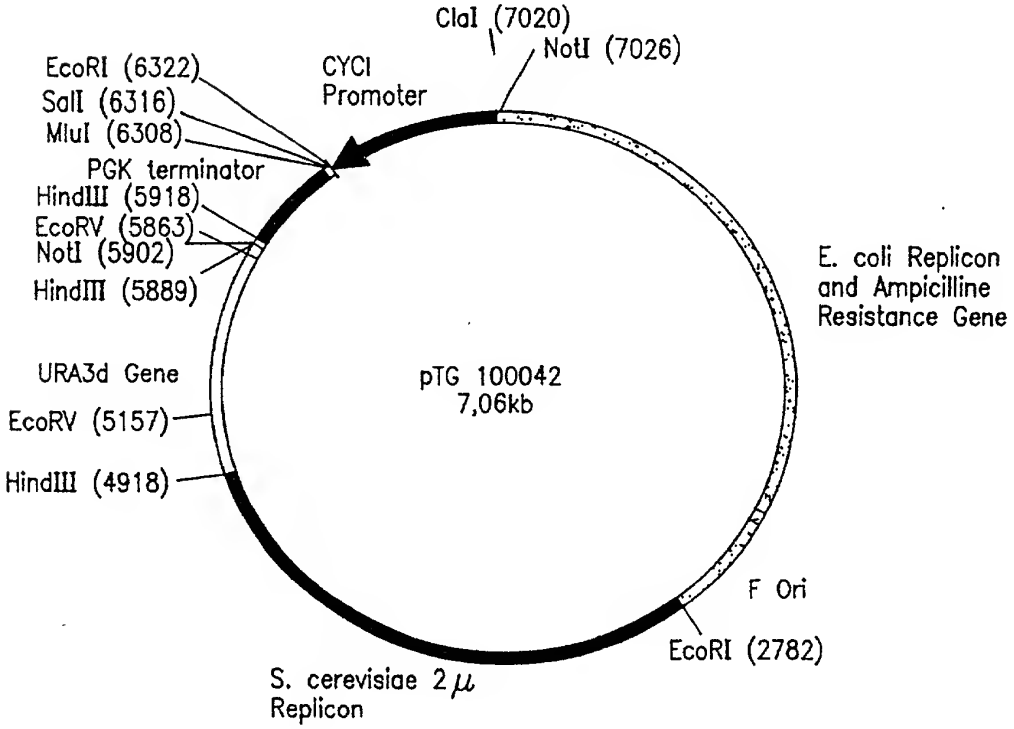


FIG. 52

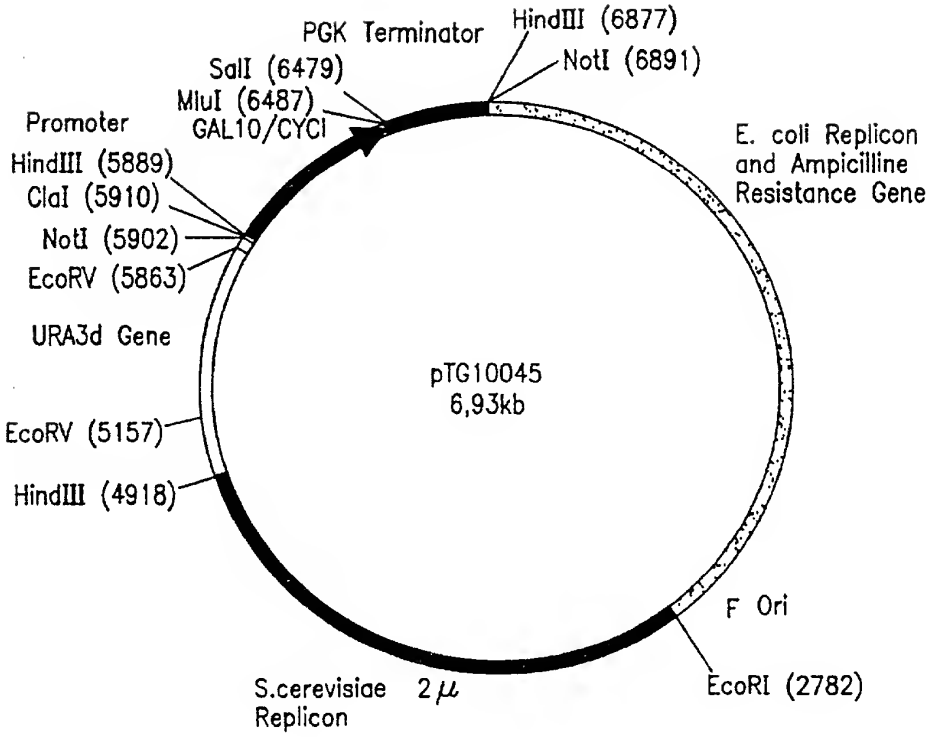


FIG. 53

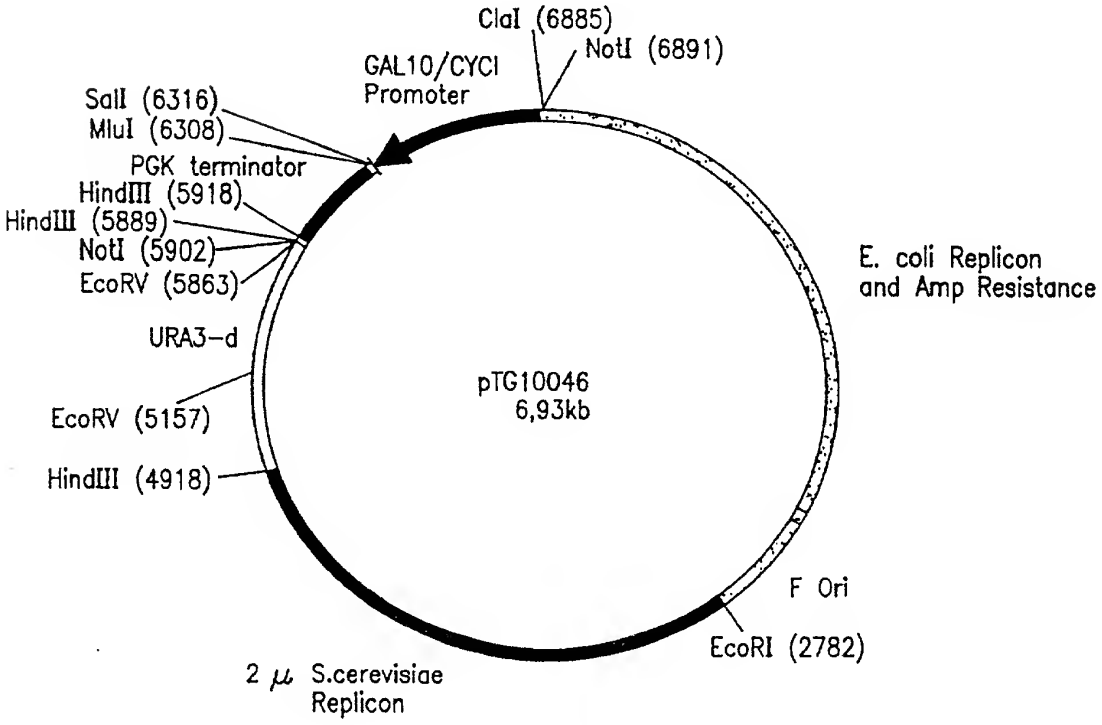


FIG. 54

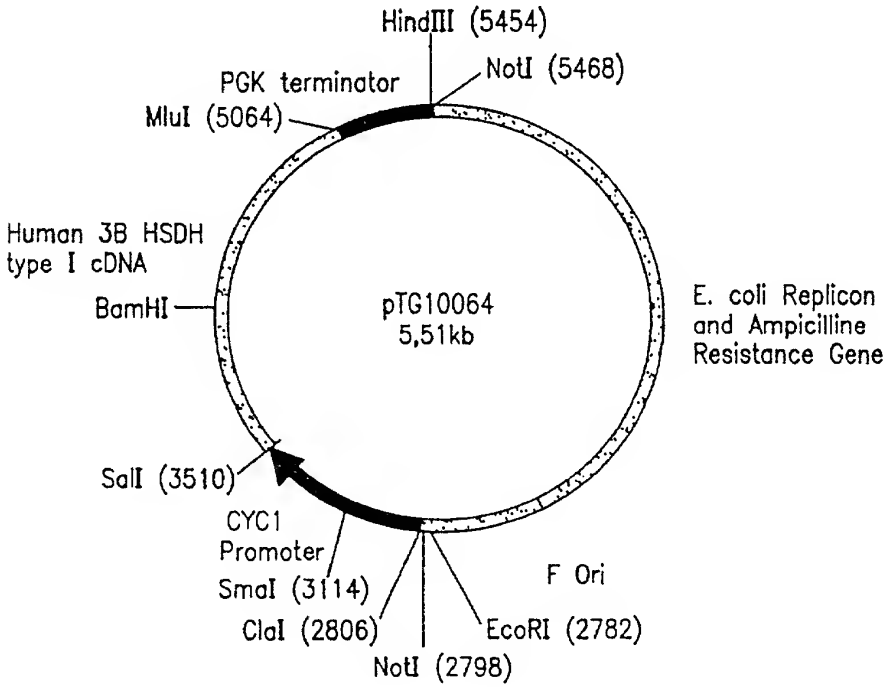


FIG. 55

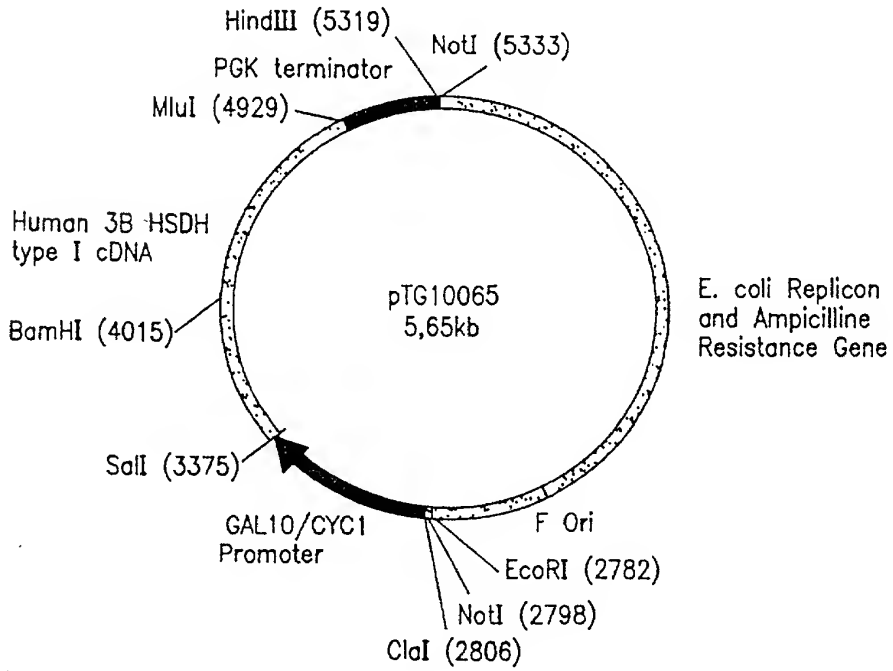


FIG. 56

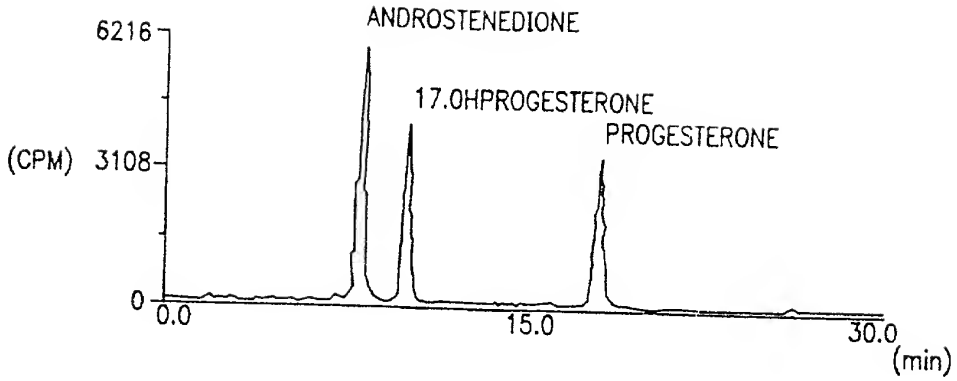


FIG. 57A

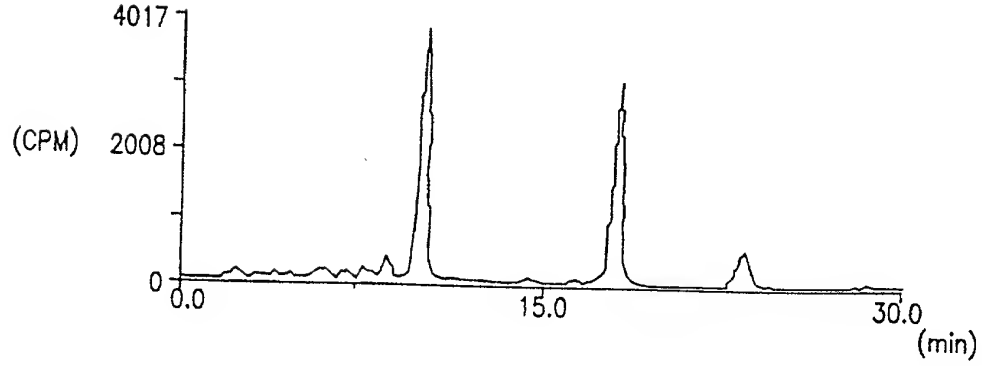


FIG. 57B